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HUMAN INNATE LYMPHOCYTES IN HOST DEFENSE, TISSUE FUNCTION AND REPRODUCTION

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Human innate lymphocytes in host defense, tissue function and reproduction
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To my family

ABSTRACT

The immune system is present in all tissues of the human body. In order to respond appropriately to infections, cells of the innate and adaptive immune system work in concert. In this thesis, we focused on natural killer cells (NK cells) and mucosa associated invariant T cells (MAIT cells), that are both present in peripheral blood and enriched in certain tissues such as the liver and, in case of NK cells, also the uterus where they are thought to be important regulators at the maternal-fetal interface. NK cells are able to react readily towards, among other things, virus-infected cells whereas MAIT cells are triggered by both cytokines in viral infections and bacterial metabolites during bacterial infections. Hepatitis C virus (HCV) is a major cause of chronic viral hepatitis and is known to impact the immune system at several instances in order to establish chronic infection. Yet, with recent advances in anti-viral treatment, the infection can now be cured in a short period of time. As a result of this, it is possible to study the impact of HCV on the immune system in a dynamic setting. Therefor we investigated both NK cells and MAIT cells in HCV before, during, and after viral clearance of the infection. Despite rapid clearance of virus, imprints inflicted by chronic HCV infection remained within NK cells and MAIT cells over time. More specifically, MAIT cells were dysfunctional, present at a reduced frequency, and displayed an altered phenotype that was only partially restored upon infection clearance. Along similar lines, also the NK cell phenotype and the diversity of the NK cell compartment remained altered after rapid HCV viral clearance. We observed persistently reduced intraindividual diversity of NK cells after elimination of chronic HCV, whereas the interindividual diversity, more linked to liver damage, was restored over time. Next, we studied peritoneal MAIT cells in ascites of patients with liver cirrhosis on the basis of viral hepatitis and other causes, and in the context of spontaneous bacterial peritonitis (SBP), a frequent and severe complication occurring in these patients. First, we could recapitulate the loss of MAIT cells in peripheral blood of cirrhosis patients in line with previous studies. Of note, in ascites, MAIT cells were more frequent than in blood and displayed a tissue-residency phenotype with increased functionality as compared to peripheral blood MAIT cells. Strikingly, during SBP, MAIT cells were the most recruited immune cells to the peritoneal cavity. Lastly, we investigated uterine NK cells (uNK cells) and hypothesized that these cells underwent a stepwise differentiation in response to endometrial regeneration and pregnancy. We present a model where uNK cells continuously differentiate throughout the menstrual cycle. This differentiation was associated with a functional shift towards immunomodulation and enhanced angiogenic function, possibly to aid in spiral artery formation during pregnancy.

In conclusion, we present data suggesting that chronic infections can leave a long-lasting imprint on the immune system. We further demonstrate that immune cell frequency, phenotype, and function can be altered depending on the respective tissue the cells reside in. This work increases our understanding of how innate lymphocytes respond to environmental cues, such as acute or chronic viral and bacterial challenges, as well as to normal physiological processes of the human body.

LIST OF SCIENTIFIC PAPERS

I. Julia Hengst*, **Benedikt Strunz***, Katja Deterding, Hans-Gustaf Ljunggren, Edwin Leeansyah, Michael P. Manns Markus Cornberg, Johan K. Sandberg, Heiner Wedemeyer* and Niklas K. Björkström*. Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. *European Journal of Immunology*, 2016, vol. 46 (9), pp. 2204-22010

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III. Christian E. Niehaus*, **Benedikt Strunz***, Martin Cornillet, Christine S. Falk, Ansgar Schnieders, Benjamin Maasoumy, Svenja Hardtke, Michael P. Manns, Anke R.M. Kraft*, Niklas K. Björkström*, Markus Cornberg*. MAIT cells are enriched and highly functional in ascites of patients with decompensated liver cirrhosis. *Hepatology*, 2020, vol. 72 (4), pp. 1378-1393

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LIST OF ABBREVIATIONS

ADCC	Antibody dependent cellular cytotoxicity
APC	Antigen presenting cell
ARC	Alcohol related cirrhosis
BCR	B cell receptor
CMV	Cytomegalovirus
DC	Dendritic cell
DEV	Donor to donor expression variation
EBV	Epstein-Barr virus
FACS	Fluorescence activated cell sorting
HCC	Hepatocellular carcinoma
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HXV	Umbrella term in this work used for viral hepatitis patients
HLA	Human leukocyte antigen
IFNγ	Interferon-γ
KIR	Killer cell immunoglobulin-like receptor
KIRL	Killer cell immunoglobulin-like receptor ligand
MAIT cell	Mucosa associated invariant T cell
MHC	Major histocompatibility complex
NK cell	Natural killer cell
PCA	Principal component analysis
PMP	Postmenopausal
SBP	Spontaneous bacterial peritonitis
SDI	Inverse Simpson diversity index, in this work used to estimate intraindividual diversity

SNE	Stochastic neighbor embedding
TCR	T cell receptor
TNF	Tumor necrosis factor
T_{reg}	Regulatory T cells
UMAP	Uniform manifold approximation and projection
uNK cell	Uterine NK cell

1 INTRODUCTION

1.1 THE IMMUNE SYSTEM

Nowadays it is known that mankind lives in close contact to innumerous microbial organisms. These can be either beneficial or, in some cases, a threat to the body's integrity. Most of bacteria and fungi live in harmony with mankind and many can colonize either our skin or internal (mucosal) surfaces. Yet, some bacteria and fungi, as well as nearly all viruses and parasites, can disrupt our body's integrity and thus threaten our health. Then it is the immune system's task to defend us. Furthermore, the immune system shields the body not only against external microbial harms, but it can also recognize and eliminate internal threats such as cancer. Recent research also points to relevant interactions between immune cells and tissues they reside in. Even if not all functions of the immune system can be mentioned here, it becomes evident that this fascinating network of cells and soluble factors is essential for human survival and wellbeing.

1.2 THE ADAPTIVE AND THE INNATE PART OF THE IMMUNE SYSTEM.

The cells of the human immune system can be classified into an adaptive and an innate branch. The adaptive arm contains T and B cells, whereas monocytes, macrophages, natural killer (NK) cells, granulocytes, dendritic cells, and mast cells belong to the innate part [1,2]. This classification derives mainly from the ability of adaptive immune cells to identify the body's own (self) and foreign structures (non-self) for which they undergo a selection process during their development [3,4]. Innate immune cells on the other hand, react immediately to certain stimuli without being primed beforehand for the discrimination between self and non-self [1]. Of course, these here described parts of the immune system work together closely and some cells, like dendritic cells, are part of the innate immune system but their main function is to interact with cells of the adaptive immune system [2].

1.3 THE ADAPTIVE IMMUNE SYSTEM

With their ability to recognize structures that do not belong to the body, adaptive immune cells are made for specifically targeting and thus eliminating those. To this end, B cells and T cells have different functions: B cells produce antibodies [5], soluble proteins that can bind to foreign structures and highlight them to other immune cells or directly neutralize them. T cells on the

other hand consist of many subsets that can, in short, either regulate the immune response or directly target cells for destruction. These functions will be described later in more detail.

The molecules recognized by the adaptive immune system are called antigens and the specific binding sites referred to as epitopes. Antigens are presented to B and T cells on the so called major histocompatibility complex (MHC) which is synonymous with the human leukocyte antigen (HLA). When the antigen is of foreign (thus microbial) origin, antigen presenting cells (APC) such as monocytes and DCs take up and process the exogenous antigens and present them on MHC class II[6]. Especially DCs play a central role in antigen presentation and T cell activation, which is why they form an important link between the innate and the adaptive part of the immune system [2]. Endogenous self antigens, such as cancerous structures, are presented on MHC class I which is expressed broadly by nearly all cells [6]. Yet, there are exceptions to the rule of self-antigens being presented on MHC class I, as some APCs are able to present also exogeneous antigens on MHC class I, a process called cross-presentation [7].

The key to this highly specific recognition of self and non-self antigens originates in the B cell receptor (BCR) and the T cell receptor (TCR) found on the respective cells. Each B or T cell expresses just one BCR or TCR. To ensure that all possible antigens are recognized, an enormous number of different T and B cells and respective receptors are necessary. To achieve this immense diversity, during B and T cell development, somatic recombination of a set of genes takes place[8]. In short, several genes can be combined in different ways so that an extremely high diversity of these receptors is possible. Some exceptions to this diversity have developed within the adaptive immune system. Unconventional T cells, to which mucosal-associated invariant T cells (MAIT cells) also belong, display a much more restricted TCR repertoire[9]. These cells and their function will be mentioned in more detail later. Thus, certain cells do not reflect all of the features of their class. As all immune cells have to work together in eliminating or controlling pathogens, contact points between the adaptive and the innate part of the immune system are crucial.

1.3.1 T cells

In the adaptive part of the immune system, T cells represent one major cell type. They can be classified according their functionality and receptor expression pattern into CD3+CD4+ T helper cells that produce immunomodulating cytokines and cytotoxic CD3+CD8+ T cells (cytotoxic T lymphocytes) which can lyse target cells directly[10]. T cells can be further

subdivided into naïve cells that have not yet encountered foreign antigen and memory T cells that already experienced activation upon antigen recognition. Memory T cells can be activated more easily and are fast to respond if they encounter the same antigen again[11,12]. Yet, also memory T cells are a heterogeneous group and can be classified according their expression of CCR7 and CD45RA in central memory (TCM), effector memory (TEM), and effector memory RA (TEMRA) [13].

Within CD4+ T cells exist subpopulations with specific features. First, regulatory T cells (T_{reg}) can be identified by their expression of CD25, the transcription factor FOXP3, and the lack of CD127[14]. As their name indicates, they are specialized in dampening immune responses and mediating tolerance[14]. Second, T follicular helper cells, marked by the expression of CXCR5 and PD-1, contribute to germinal center reactions promoting B cell maturation, but can also be found in periphery[15].

1.3.2 Unconventional T cells

While conventional T cells are able to recognize a great variety of possible antigens, unconventional T cells, such as NKT cells, γδT cells, and MAIT cells, are restricted in their TCR repertoire and thus in their recognition of antigen [9]. They are able to exert effector functions faster than conventional T cells and to respond to molecules other than peptides, but do not form memory cells [9]. NKT cells respond among others to bacterial lipids presented on the CD1d molecule [16], indicating that they are able to respond to bacterial infections. In line, also γδT cells recognize microbe originating molecules [17] but also get activated by cellular stress signals such as MICA and ULBPs [18,19]. Thus, unconventional T cells appear to take over functions that are typically more associated with the innate immune system and represent a faster response against pathogens.

1.3.3 MAIT cells

Belonging to unconventional T cells, MAIT cells uniformly express the TCR Vα7.2 segment and possess furthermore only a limited TCR Vβ chain repertoire [20,21]. The antigens recognized by this rather restricted TCR repertoire are predominantly riboflavin metabolites that are presented by the MHC class 1-like molecule MR1[21,22]. As these metabolites derive from bacteria or fungi, the assumed main functional role of MAIT cells is to respond to bacterially infected cells [22,23]. Yet, MAIT cells are not limited to bacterial infections in their response as

they are also able to recognize fungal organisms [24] and be activated by cytokines such as IL-7, IL-12, and IL-18 typically produced during viral infections [21,25,26]. Another identifying feature of MAIT cells is the expression of CD161 [27]. This receptor (previously also called NKR-P1A) was discovered to be expressed on NK cells and a subset of T cells [28] and appears to correlate to a "innate-like" signature [29]. Thus, despite belonging to adaptive T cells, MAIT cells can also be referred to as innate, or innate-like T cells, given their limited TCR repertoire and the innate effector functions they acquire already early in life [30].

Even though present in peripheral blood, MAIT cells, as their name suggests, are enriched in mucosal surfaces [20], but even more so in the liver where they can represent up to 50% of all T cells [27]. For a yet unknown reason, especially during chronic infections [31,32] or inflammatory conditions [33,34] the abundance of MAIT cells can drop dramatically. The relevance of this loss of MAIT cells is currently unknown, but it is hypothesized that in chronic infections this loss might render patients more susceptible to additional infections and therefor adds to the burden on the immune system in chronically infected patients[35].

MAIT cells in viral infections

Despite their predisposition to react during bacterial infections, MAIT cells are contributing also to anti-viral immunity. This has been studied for example in influenza, where higher MAIT cell numbers correlate with clinical outcome [36] but also in murine models where they protected from lethal disease [37]. Additionally, MAIT cells are also activated in other viral infections, such as those by dengue virus [38] and HIV [31,39], and thought to contribute to the immune response. As these viruses do not share common features but instead represent quite distinct families, it appears that MAIT cells are a common part of the immune response towards viral infections.

1.4 THE INNATE IMMUNE SYSTEM

As already mentioned, the human immune system consists also of cells that do not undergo a development to distinguish self and non-self structures. These cells form the innate immune system. Yet, it would be wrong to say that the innate immune system cannot tell apart foreign and the body's own antigens, as innate immune cells can express different receptors, as for example the pattern-recognition receptors, that react specifically to bacterial or viral structures[40]. The difference is more that adaptive immune cells have a variety of possible

antigen receptors and undergo selection to discern the body's own and foreign structures whereas innate immune cells express the same kind of receptors without selection.

1.4.1 NK cells

Belonging to innate immune cells, one key feature of NK cells is their ability to lyse target cells directly and without preceding sensitization, hence their name "natural killer cells". They represent roughly 10 % of all lymphocytes in circulation and are defined as CD56+CD3- lymphocytes that can be subdivided according their expression level of CD56 into CD56dim and CD56bright NK cells[41]. The function of CD56 itself is still topic of current research, though it appears to be relevant for their ability to form a lytic synapse when lysing target cells [42]. The afore mentioned subsets, CD56dim and CD56bright NK cells, also differ in their expression of the Fc receptor CD16, which is highly expressed on CD56dim but found only at low levels on CD56bright NK cells[41]. This results also in different functional capacities, as CD56dim NK cells are more potent in target cell lysis and antibody-dependent cytotoxicity (ADCC) whereas CD56bright NK cells produce more cytokines and have a higher proliferative potential[41].

The function of NK cells is regulated by a variety of inhibitory and activating receptors. Common activation receptors are CD16, NKp46, NKp44, NKp30, DNAM-1 and NKG2D, whereas inhibitory receptors are NKG2A, LILRB1 and most of the killer cell immunoglobulin-like receptors (KIR) [41,43]. The expression pattern and the levels of these are important, as the resulting balance of activating and inhibiting signals determines NK cell function [44]. A unique feature of NK cells is that some of these inhibitory receptors are able to recognize MHC on target cells. Thus, a lack of MHC on a target cell leads to activation of the NK cell, a mechanism which is called "missing-self" recognition [45]. The biological significance becomes evident in the case of tumors or infected cells: downregulation of MHC might enable escape from T cell mediated lysis, but NK cells would then sense this "missing-self", become activated and lyse the target cell.

KIR receptors

KIR receptors are expressed mainly on NK cells and are single immunoglobulin chains that can transmit either inhibitory or activating signals[46,47]. They recognize parts of the MHC class 1 (in this context referred to as KIR-ligands (KIRL)) and are thus crucial for the NK cells' ability to recognize the absence of MHC[45,48].

Similar to the MHC system, also KIR receptors are encoded by highly diverse genes and each individual person has a specific genotype[49,50]. According to the given genotype of KIRs, two main haplotypes can be distinguished: KIR haplotype A which only consists of the combination KIR2DL1, -2DL3, -2DL4, -2DP1, -2DS4, -3DL1, -3DL2, -3DL3 and -3DP1, whereas all other KIR genotypes belong to KIR haplotype B [51,52]. Within the haplotypes one can further differentiate according to gene localization on chromosome 19: whereas KIR haplotype A always has centromeric and telomeric A, a KIR haplotype B could be either centromeric A/telomericB, centromeric B/telomeric A, or centromeric B/telomeric B[53]. This is of functional importance, as for example the telomeric haplotype B has been associated with reduced CMV reactivation after kidney transplantation[54] and a centromeric B with protection from preeclampsia[55]. Yet it is not only the KIR and their pattern that matter but also their cognate ligands. For HLA-C there exist two allotypes that are recognized: HLA-C1 which is bound by KIR2DL2/-2DL3 and HLA-C2 bound by KIR2DL1 and -2DS1[56]. Further known ligands are the HLA-B allotypes Bw4 for KIR3DL1 and HLA-G for KIR2DL4[56]. The relevance of the KIR-KIRL interaction is highlighted in hepatitis C virus infection (HCV), where it has been shown that the combination of KIR2DL3 with a homozygous C1/C1 allotype correlates with clearance of the virus[57]. Except for this, in the last years, many associations between KIR-KIRL combinations and disease outcomes have been identified[58]. This not only implies that NK cells in some way are involved in these conditions but also that the KIR-KIRL system is a crucial regulating element.

NK cell maturation, licensing, and expansions

In investigations on the impact of KIR and KIRL on NK cell functionality, a phenomenon known as “licensing” or “education” was uncovered. If NK cell development takes place with a KIR present on the NK cells and its cognate KIRL expressed by the host, these NK cells become “licensed” and are eventually more potent in lysing MHC-deficient NK cells and cytokine production[59-61] compared to NK cells positive for KIR without a cognate KIRL expressed by the host. This process on the other hand is uncoupled from NK cell maturation. NK cells in peripheral blood differentiate from KIR-CD57-NKG2A+ CD56bright NK cells over CD56dim cells that acquire CD57, downregulate NKG2A expression to finally become CD56dim KIR+CD57+NKG2A-[62]. Yet this might not hold true for tissue residing cells, as the phenotype of NK cells in tissues is not always compatible with this model established for peripheral blood [63,64].

One viral infection that is highly relevant for NK cells and can substantially imprint this compartment is cytomegalovirus (CMV) infection. It has been shown that NK cells acquire adaptive features that after the first exposure would protect from CMV in a murine setting [65]. Furthermore, in humans, the NK cells' KIR haplotype correlates with the frequency of CMV reactivations after stem cell transplantation[66]. This indicates that NK cells are crucial for control of CMV infection. Subsequently, it became evident that human NK cells would in roughly 30% of all CMV infections develop expansions with a distinct phenotype of NKG2C+ CD56dim NK cells which express highly selective KIRs and readily respond to CMV infected cells [67,68]. These expansions are very stable over time, appear to be affected by strain specific peptides [69] and can be linked to epigenetic modifications of the NK cells[70].

1.5 LYMPHOCYTES IN CIRCULATION AND IN TISSUES

In the recent years, more focus has been shifted towards immune cells that are tissue resident. In anatomy, one distinguishes between circulation (that is peripheral arterial and venous blood), primary lymphoid organs (thymus and bone marrow) and the secondary lymphoid organs (lymph nodes and spleen). In the tissue, lymphoid fluid containing also immune cells is drained by lymph vessels via several lymph nodes and finally reentering blood circulation. Previously, the majority of immunological research had been done on lymphocytes from peripheral blood, mostly because of constraints in access to peripheral tissues. Within a tissue, resident immune cells can be identified by expression of CD49a, CD103, as well as the C-type lectin CD69 [63,71]. CD49a and CD103 are integrins that bind extracellular collagen [72] or E-cadherin on epithelial cells [73], respectively, whereas CD69 acts as inhibitor of sphingosine 1-phosphate receptor-1 that mediates lymphocyte egress from tissues [74,75]. Thus, these markers act together in retaining lymphocytes within peripheral tissues. With respect to CD8 T cells, tissue resident populations have been reported that are retained in tissues and do not recirculate to peripheral blood [76]. This is of interest, as one can hypothesize that certain cells primarily participate in local immune response whereas others surveil circulation.

1.6 THE UTERUS

The human reproduction poses a dilemma to the immune system: On one hand, during pregnancy, the contact of the fetus, via the placenta, to the maternal side is essential, even though fetal antigens could be recognized as non-self and rejected. On the other hand, infections in the female reproduction tract need to be identified and cleared. All this takes place in the uterus and the residing immune cells thus have to tolerate to a certain degree non-self

tissue during pregnancy, to follow the uterus' changes throughout the menstrual cycle and pregnancy and still be able to protect it from infections.

To start with, the uterus itself consists of three layers – the perimetrium, the outer layer covering it, the myometrium which is the muscular layer in the middle and as inner lining the endometrium, the mucosal surface of the uterus. From an immunological point of view, especially the endometrium is of interest as it is here where the fertilized egg first implants and the essential contact is created. In pregnancy, the endometrium differentiates into the decidua, the maternal part of the placenta.

After implantation, cells from the outer layer of the embryo differentiate into the so-called villous trophoblast (VT), covering the fetal villi that are in contact with the maternal blood in the placenta, and the extra-villous trophoblast (EVT) that migrates into the maternal tissue and participates in arterial remodeling [77]. Thus, the trophoblast cells are crucial in creating the maternal-fetal interface, enabling exchange of oxygen and nutrients between the maternal and fetal side and creating a tolerogenic environment [78,79]. Because the fetal-derived trophoblast cells interact with maternal immune cells they have evolved unique adaptations. To this end, trophoblast cells express a unique profile of HLA molecules that minimizes T cell recognition, with VT not expressing any HLA and EVT lacking selectively HLA-A, -B, and HLA class II [80]. In addition, further ways to suppress immune reactions are described such as production of TGFβ and VIP [81,82]. Those HLA that are expressed by EVT are recognized by NK cell receptors, indicating a possibly skewed interaction between EVT and immune cells towards tissue residing NK cells [83].

1.6.1 Uterine NK cells

The hypothesized interaction between trophoblast and NK cells is strengthened by the fact that NK cells are found in the uterus in frequencies exceeding those in peripheral blood [64]. NK cells are already present in the endometrium at a high frequency and then increase in numbers in early pregnancy in the decidua[84,85]. Not only do NK cell frequencies differ from peripheral blood but NK cells residing in the uterus also display a distinct phenotype [84]. These so called uterine NK cells (uNK cells) express high levels of NKG2A, are negative for CD16 and CD57 but express a high percentage of KIR [64]. In contrast, in peripheral blood such a phenotype with the duality of KIR expression and markers indicating an immature phenotype is nearly non-existent[86].

Studying the KIR receptors on NK cells, there are correlations between KIR-KIRL interactions and pregnancy disorders such as preeclampsia [87] and low birth weight [88]. Notably, uNK cells express KIRs at different frequencies and combinations than conventional NK cells (cNK cells) in peripheral blood [89,90]. The KIRs expressed are also further skewed towards expressing more KIR2DL isoforms and less KIR3DL1 [86,91]. The resulting function of uNK cells seems to be linked to the trophoblast invasion and regulation of placental blood supply [92], but it has also been suggested that they might secrete factors that directly influence fetal growth [93]. In summary, uNK cells are specialized for the uterine environment and the interaction with the fetal side and appear to be pivotal in the uterine immune system. However, many aspects of uNK cell biology remains elusive including if these cells undergo any type of differentiation in response to endometrial regeneration and decidual transformation.

1.7 THE LIVER, ITS IMMUNE SYSTEM AND ITS PATHOLOGIES

The liver is an organ with a variety of functions including storing nutrients, clearing potentially hazardous agents, and producing molecules part of inflammation/infection control, such as C reactive protein (CRP). Some of these functions, such as storage of nutrients, can be explained by the liver's anatomy, as most of the blood from the intestine first passes from the portal vein through the liver before continuing its journey throughout circulation. Thus, the liver is exposed to everything that is passed on to the blood from the intestine. Potentially hazardous agents are then cleared by the liver to the bile, which ends in the small intestine and finally get excreted through feces.

Furthermore, the portal venous blood flow also leads to immune cells in the liver coming in contact with antigens from the gut. In addition, harms to the liver need also to be handled by the local immune system. Therefor the liver is not only an important site in metabolism but also in immunology as both tolerance but also immune reactions can be initiated here [94]. To this end, it harbors a special composition of immune cells: among T cells, MAIT and γδT cells are more abundant [27,95], but also NK cells are more frequent than in blood [96,97]. Additionally, liver resident NK cells possess a distinguished phenotype as $CD16^-$ $CXCR6^+$ $CD69^+$ $CD49e^-$ NK cells [98,99], that is present only at low frequency in peripheral blood. Within the myeloid compartment, there are specialized macrophages, so called Kupffer cells, that are resident in the liver [100], and other monocytes that can be recruited from peripheral blood [101]. Thus, it becomes clear that in the liver a certain immunological milieu is created in order to maintain tissue function and to mount an adequate immune response.

1.7.1 Viral infections of the liver

There are many pathologies that involve the liver, either primarily or indirectly. Yet, the majority of all chronic liver pathologies derives either from primarily liver affecting viruses (hepatotropic viruses) such as hepatitis B and C virus (HBV and HCV) or inflammatory conditions like autoimmune hepatitis [102] and alcoholic- and non-alcoholic steatohepatitis (ASH and NASH, respectively) [103,104]. Viral infections can harm the liver either directly, as the virus infects liver cells (hepatocytes) and can in consequence induces cell death, or indirectly through immune cells that recognize and lyse infected cells. Furthermore, one can distinguish viruses that cause primarily acute infections (hepatitis A and E) or those that are prone to establishing chronic infections, such as HBV, HCV, and hepatitis D (HDV). The latter, HDV, is not able to replicate by itself, instead it needs a co-infection with HBV (either prior to HDV contact or simultaneously) [105]. Notably, HBV and HCV make up the majority of all chronic viral hepatitis infections [106]. Yet, they derive from different virus-families and in consequence there are profound differences between these two infections [107]. While HBV is frequently vertically transmitted and leads in this setting nearly always to chronic infection, in adults the majority of infected patients recover [108]. In contrast, in HCV, vertical transmission is less frequent and adult infections become chronic in most cases [108,109].

1.7.2 Hepatitis C virus

HCV is a single stranded RNA virus that often causes chronic infection[110,111]. A chronic infection with HCV can ultimately, in selected patients, lead to liver cirrhosis and development of hepatocellular carcinoma (HCC) [112]. Hepatitis C virus can be classified into six genotypes and genotype distribution varies geographically. The most prevalent genotypes in Europe and North America are genotypes 1 and 3, whereas genotype 4 is more prevalent in North Africa and Middle East[113]. These different genotypes are important to determine since they influence the disease outcome [114].

In order to establish a chronic infection in first place, HCV has mechanisms to undermine and hamper the immune response. For example, CD8+ T cells can progress into an exhausted state which is marked by profound phenotypic and functional changes. These exhausted T cells display reduced ability to proliferate [115] and lower production of cytokines [116]. They also show upregulation of inhibitory receptors such as PD-1, TIM-3, and CTLA-4[117-120], all of which reduce the functionality of HCV specific T cells. Of interest, blocking the inhibitory signals

can restore T cell functionality [119], indicating that some of these the observed alterations can be restored.

There is evidence for NK cells taking part in control of HCV infection as a specific KIR-KIRL genotype, KIR2DL3 and homozygosity for HLA-C1/C1, strongly correlates with resolution of HCV infection [57]. Therefor it is of interest, that HCV can interfere also with NK cell activation and function, either via alteration of monocyte function that in turn influences NK cells or via direct interaction with infected cells [121,122]. Lastly, not only NK cells and CD8+ T cells are affected, but also other immune cells are influenced, as for example altered T_{reg} [123] and DC frequencies[124] have been reported. Thus, HCV infection has a profound impact on the immune system which makes it clear why this pathogen is so successful in establishing chronic infection.

From a clinical point of view, treatment of chronic HCV has undergone a revolution in recent years from a hard-to-treat disease to currently a >95% cure rate [125]. The newly developed drugs, referred to as direct acting antivirals (DAA), cure HCV within a short period of time [107,125]. This rapid clearance makes it possible to answer questions regarding the immunology of hepatitis C, for example if the immune dysfunction can be restored or if an imprint on the immune system remains.

1.7.3 Liver cirrhosis

Eventually, all chronic liver diseases can lead to increased fibrosis and finally liver cirrhosis [126]. This final step of the diseased liver is thought to be irreversible and comes with high mortality [127]. Cirrhosis itself is defined by a fibrotic reorganization with loss of regular liver structure, smaller size of the liver and heightened stiffness which can be diagnosed either by histopathology, ultrasound, or transient elastography (e.g. FibroScan®) [126,128]. This stiffness and increased fibrosis can lead to a higher pressure in the blood flow to the liver, thus called portal hypertension. The higher pressure in this area can in turn lead to circumventions via veins that become enlarged (varices) and have a higher risk of bleeding [126]. Additionally, fluid accumulation in the peritoneal cavity (ascites) because of portal hypertension can reach significant amounts and crave medical intervention [126]. Depending on the presence of cirrhosis related complications, one speaks either of “compensated”, when otherwise asymptomatic, or “decompensated” cirrhosis, when complications such as liver dysfunction, ascites, or varices occur [127].

Apart from more localized effect, cirrhosis leads also to systemic perturbations. For example, dysfunction of the liver and its anatomical changes can lead to cirrhosis-related immune dysfunction [129]. Thus, it is not surprising, that a significant proportion of the cirrhosis-related mortality is due to infections [130]. In line, the combination of immune dysfunction and higher pressure in the portal vein and ascites, it is not surprising that bacterial translocations can occur from the gut to the ascites. This then leads to a severe infection of the peritoneal cavity, a so called spontaneous bacterial peritonitis (SBP). Not only is SBP the most frequent infection in cirrhosis [131], but also causes high mortality [130]. If and how the immune dysfunction in patients with liver cirrhosis predisposes to acquisition of SBP and increased mortality is not yet known.

2 AIMS

The overall aim in this work was to assess the impact of chronic infection and/or inflammation as well as tissue microenvironments on the shape of the human immune system with a focus on NK cells and MAIT cells. The specific aims addressed in each project are as follows:

2.1 DEFINING THE IMPACT OF CHRONIC HCV INFECTION ON INNATE LYMPHOCYTES AND UNCONVENTIONAL T CELLS

2.1.1 Analysis of MAIT cells before and after clearance of HCV (project 1)

2.1.2 Study of the NK cell compartment and its diversity in DAA mediated viral clearance (project 2)

2.2 INVESTIGATION OF THE PERITONEAL IMMUNE SYSTEM IN LIVER CIRRHOSIS WITH A FOCUS ON MAIT CELLS (PROJECT 3)

2.3 ESTABLISH A MAP FOR HOW UTERINE NK CELLS DIFFERENTIATE IN RESPONSE TO ENDOMETRIAL REGENERATION AND PREGNANCY (PROJECT 4)

3 METHODS

3.1 ETHICAL CONSIDERATIONS

Ethical evaluation must be the basis for all medical research. In each project, ethical evaluation took place before start of the study and ethical permissions were granted from the relevant ethical authorities.

In the case of adult HCV patients, peripheral blood was sampled (Permit numbers for Project 1 &2: 2148-2014). Healthy adults as part of control cohorts were either acquired by sampling peripheral blood or using buffy coats which are prepared remnants after blood donation (Project 1&2: 2148-2014, Project 4: 2006/229-31/3). Matched peripheral blood and ascites samples from cirrhosis patients or peripheral blood samples for controls was used in Project 3 (permit number: 3188-2016). In Project 4 peripheral blood and endometrial samples were used (2013/1324-31/2 with 2014/1996-32 and 2016/1313-32), as well as samples from decidua (2012/1968-31/1 and 2017/649-31/1). Other cohorts included were endometrial samples from uterus transplantation (362-16 and T1052-16), monozygotic twins (2013/1324-31/2 and 2017/193-32) as well as control samples from tonsils (2006/646-31/4, 2013/217-21, 2014-1470-32-4 and 2015/1083-32) and liver (2013/2285-31/3). Animal experiments were performed after approval by the Linköping Animal Experimentation Ethics Committee (2015/1368-31/4, 2015/2122-32 and 29-15).

To conclude, for all projects ethical evaluation took place, the necessary permissions for sample acquisition and analysis have been granted and all patients gave written consent prior to study inclusion.

3.2 COLLECTION OF SAMPLES AND PROCESSING

Peripheral blood samples were collected in heparin-coated tubes via venous puncture. The blood of each donor/patient was pooled and PBMC isolated via density-gradient centrifugation. After collecting the interphase containing the PBMC, the samples were washed and either used for experiments or frozen down at -180°C in FCS with 10% DMSO for long-term storage **(Paper 1-3**).

Tissue samples from the uterus were subjected to mechanical dissection with scalpels before enzymatic digestion at 37°C with Collagenase II (Sigma) and DNase I (Sigma). After that, mononuclear cells (MNC) were isolate via density gradient centrifugation and frozen as

described above **(Paper 4**). Liver samples were first flushed to remove excess blood before being perfused with collagenase XI for enzymatic digestion and finally mononuclear cells were isolated and frozen as above **(Paper 2 and 4**). Tonsil samples were mechanically dissected and filtered through a 40μm mesh before being frozen as above **(Paper 4**). Ascites cells were centrifuged and subjected to red blood cell lysis before being frozen for long term storage **(Paper 3**). Menstrual blood was collected as described before [86]. In brief, the donors collected the blood in menstrual cups before being transferred to collection medium containing heparin, amphotericin B and penicillin/streptomycin. The blood was then processed and frozen as described for peripheral blood **(Paper 4**).

3.3 FLOW CYTOMETRY AND CELL SORTING

One of the main methods in immunology is flow cytometry, or fluorescence-activated cell sorting (FACS). For this analysis, cells are stained with fluorochrome-conjugated antibodies, before being acquired on the flow cytometer. Here, the emissions of these fluorochrome-antibody-conjugates after laser excitation are recorded and the expression of molecules can be analyzed.

To this end, the either untreated cells (for *ex vivo* phenotyping) or stimulated cells (for assessment of functionality) were stained with a combination of antibodies and dead cell marker (eBioscience). Then, cells were fixed and, if an intracellular staining was to be performed, permeabilized. Intracellular staining was necessary for recording of transcription factors or other intracellular molecules, such as interferon-γ (IFNγ) and TNF.

After acquisition, flow cytometry files were analyzed in FlowJo version 9 **(Paper 1-4**) and FlowJo version 10 **(Paper 3-4**). Here, experimental sets were compensated and populations of interest gated and marker expression analyzed. Dimensionality reducing methods such as stochastic neighbor embedding (SNE) and uniform manifold approximation and projection (UMAP) analysis were used either in R version 3.3-3.6 or as plug-in of FlowJo version 10, Phenograph clustering analysis was used as plug-in of FlowJo version 10. For these analyzes, of the populations to be investigated, the same amounts of cells were exported and barcoded to enable back tracing.

In some experiments, the function or transcriptome of a specific subset of immune cells was of interest. Here, cells were stained without fixation and then sorted alive into the desired subset on a BD FACSAria, FACSAria Fusion, or a Sony MA900 cell sorter **(Paper 4**).

3.4 FUNCTIONAL ASSAYS

For studies of peripheral blood NK cells, established assays to assess natural cytotoxicity, ADCC, and cytokine response were used. To this end, NK cells were thawed, plated and rested overnight. Natural cytotoxicity was investigated via six hours incubation with K562 target cells at an effector to target of 10:1 and ADCC after incubation with 721.221 cells with the anti-CD20 antibody Rituximab. Response to cytokines was examined via stimulation overnight with IL-12 /IL-18 at 10ng/mL and 100ng/mL, respectively **(Paper 2**).

Functionality of MAIT cells both in peripheral blood and in ascites was assessed both with stimulation via the MR1-TCR axis and cytokine response **(Paper 1** and **3**). For TCR stimulation, *E. coli* from the laboratory strains D21 or DH5α were fixed shortly before being added to plated PBMC. The cytokine stimulation of MAIT cells was performed in the same way as for NK cells **(Paper 1 and 3**).

In all functional assays for flow cytometric analysis, Brefeldin A and Monensin (GolgiPlug/Stop, BD Biosciences) were added for the last 5 hours. At the end of the assay, cells were stained with fluorescently-labelled antibodies and fixed before acquisition on the flow cytometer.

Investigation of uNK cell function **(Paper 4**) was either performed via generation of supernatants or direct stimulation for 6 hours with PMA/Ionomycin (10ng/mL and 500nM, respectively) for flow cytometric analysis. Supernatants were collected after stimulation with IL-12/IL-15/IL-18 overnight at 20ng/mL, 20ng/mL and 200ng/mL, respectively, or with PMA/Ionomycin as described above. Supernatants were collected after centrifugation and frozen at -80°C until further use. The soluble factor profile of supernatants were then analyzed by SciLife, Uppsala, via Proximity extension assay (PEA), a method using oligonucleotide-tagged antibodies for detecting up to 92 proteins simultaneously.

Proliferation of uNK cell subsets was investigated after plating of equal numbers of CellTrace labelled sorted uNK cells and stimulation with IL-15 at 10ng/mL for seven days. Medium was replaced every other day. For *in vivo* differentiation assays, sorted uNK cell subsets were injected intravenously in MISTRG mice together with intraperitoneal IL-15 (at day 0 and day 5); after seven days mice were sacrificed and cells from uterus, spleen and liver were analyzed.

3.5 RNA SEQUENCING

For investigation of the transcriptome of uNK cell subsets, the respective subsets were sorted into RLT buffer (Qiagen). Purification of RNA, subsequent cDNA preparation as well as sequencing was performed by the Bioinformatics and Expression Analysis (BEA) core facility, Huddinge. In brief, transcriptome data was then analyzed after quality control with FastQC (v0.11.5), trimming via Trim Galore! (v0.6.4) alignment to the reference Genome with STAR (v020201) and finally DESeq2 package (v1.22.2) for differentially expressed genes (DEGs). For gene set enrichment analysis (GSEA), either DEGs were compared to the background list with the GOrilla tool [132] or the ranked list of expressed genes was compared to publicly available databases [133,134] with the GSEA software tool (v4.0.1) [135,136].

3.6 MICROSCOPY

Imaging of uterine samples (either endometrial or decidual, **paper 4**) was performed sections from OCT embedded snap frozen samples. Expression of CD39, Eomes, CD3, and DAPI was visualized via Confocal Microscopy. To this end, samples were fixed, blocked to protect unspecific antibody binding before being incubated first with primary and then secondary antibodies. Imaging was performed on a Nikon A1R confocal microscopy instrument and images analyzed with the Imaris software.

3.7 STATISTICS

Data was analyzed in Graphpad Prism versions 6-8. In a first step data was tested for normal distribution. If not-normally distributed, either a Wilcoxon, Mann-Whitney, Friedman's or Kruskal-Wallis test or Spearman correlation were performed, depending on number of sample groups and if paired samples should be compared. In case of normal distribution, either paired/unpaired t-test, or Pearson correlation were chosen. Fisher's exact test was used for contingency tables.

3.8 ADDITIONAL RESOURCES

Further analysis, such as principal component analysis (PCA), were performed in R (versions 3.5.3 and 3.6.0) with packages factoextra (v1.0.5). Certain figures were created with BioRender.com.

4 RESULTS AND DISCUSSION

4.1 THE IMPACT OF CHRONIC HCV INFECTION ON THE IMMUNE SYSTEM

Chronic infection with HCV leads to severe alterations in the immune system [108]. In T cells, it has been well described that chronic HCV infection can cause immune exhaustion. These HCV specific and exhausted T cells display elevated expression of inhibitory receptors, such as PD-1, reduced proliferative capacity, and weak cytokine response to antigen stimulation[115-117]. In the innate immune system, NK cells have been shown to be important in spontaneous resolution of HCV infection [57] but to be dysfunctional in chronic disease [137,138]. Thus, HCV is able to evade the immune system by suppressing and altering its functionality at different levels. The recent approval of DAA for the treatment of HCV has drastically improved its prognosis and HCV can now be cured within a short period of time [125]. This allows to study the immune system in HCV in a dynamic setting where the chronic infection is rapidly removed and one can investigate if immune dysfunction can be restored or if it there is a lasting effect. Thus, in collaboration with the medical university of Hannover, patient and control samples were collected and studied for MAIT and NK cell phenotype and function before, during and after viral clearance **(Paper 1 and 2**).

4.1.1 MAIT cell frequencies are reduced in chronic HCV infection

It has been shown that MAIT cells can be activated in viral infections [25,36], but that their abundance in peripheral blood during these infections is significantly diminished, as for example in chronic HIV [31]. Therefore, we set out to investigate MAIT cells in the setting of chronic HCV infection and subsequent viral clearance upon DAA treatment **(Paper 1**). In order to get a broader picture of the immune system, we first determined the percentages of 26 immune cell populations in peripheral blood of chronic HCV-patients and healthy controls **(Paper 1, Figure 1**). In comparison to healthy controls, we observed alterations that have been described before, such as elevation of memory T cells [139] and T_{reg} [123]. Yet, the most pronounced change was a loss of MAIT cells in periphery from an average of 3.4% of T cells in healthy controls to 0.6% in HCV patients. Of note, this loss was confined to MAIT cells, as the T cell population expressing TCRVα7.2 but lacking CD161 displayed similar percentages in patients as in healthy controls. To see if the loss of MAIT cells could be linked to other alterations in the immune system, we correlated the percentages of the respective immune cell populations. Here, we found no significant correlations between MAIT cells and other immune cell types, indicating that this is a more isolated effect of

chronic HCV on the MAIT cell population. Altogether, we noted a significant reduction of MAIT cells in chronic HCV.

4.1.2 Persistent reduction of MAIT cell abundance and continioued dysfunction upon HCV clearance

As the noted loss of MAIT cells represents the state of chronic infection before initiation of antiviral therapy, it was of interest to investigate if this impact of HCV infection on the immune system could be restored after viral clearance. It has been shown that reduced MAIT cell frequencies are reversible in certain conditions, for example as the loss of MAIT cells in obese patients can be reversed after bariatric surgery and ensuing weight loss [33]. Furthermore, it has been reported that T cell dysfunction in HCV to some extent is restored after treatment with DAA and viral clearance [140]. Thus, it could be speculated that also MAIT cell alterations are reversible upon elimination of the chronic insult. Here, we first investigated the dynamics of viral clearance in our patient cohort and could recapitulate the previously observed rapid drop of HCV RNA levels during DAA treatment [125,141] **(Paper 1, Figure 2**). Of interest, despite this rapid resolution of HCV infection, MAIT cell numbers remained unaltered, even up to 72 weeks after start of treatment. Looking at the phenotype of MAIT cells in chronic HCV infection, we observed an activated phenotype with elevated expression of granzyme B, CD69, HLA-DR, and PD-1. In line with the persistent reduction in MAIT cell frequencies, we noted only a partial restoration of the MAIT cell phenotype with prolonged elevated levels of activation markers.

Given the altered abundance and phenotype of MAIT cells, we next studied if also the functionality of MAIT cells was altered in chronic HCV infection **(Paper 1, Figure 3**). Upon stimulation with *E. coli,* MAIT cells from HCV patients displayed lower levels of granzyme B, TNF and IFNγ production and reduced degranulation (indicated by CD107a). In agreement with the observed persistence with respect to changes in frequency and phenotype, this observed dysfunction was not restored after viral clearance. Looking at cytokine priming of MAIT cells, surprisingly no changes in MAIT cell function could be observed in chronic infection. Thus, only TCR-dependent stimulation appeared permanently affected in MAIT cells while their ability to respond to cytokines was not. Lastly, also on the transcription factor level, alterations were noted. MAIT cells from HCV-patients expressed less PLZF, a main driver of effector functions [142]. Even though we could not determine a direct correlation between PLZF and MAIT cell function, the lower expression levels might be relevant for the noted dysfunction.

Concluding, despite rapid viral clearance after DAA treatment we found persistently reduced MAIT cell frequency and irreversible dysfunction in remaining MAIT cells in chronic HCV. A limitation of our study is the fact that we analyzed MAIT cells only from peripheral blood and not the liver, yet more recent studies could recapitulate our findings in peripheral blood and also in intrahepatic MAIT cells [143,144]. Furthermore, similar patterns had been observed in chronic HIV infection, where MAIT cell numbers remained reduced and their phenotype and function partially restored, even if patients were und antiviral treatment and virus replication suppressed [21]. The reason for this loss in MAIT cells is not yet known. It might be the constant stimulation in chronic infections that is driving increased MAIT cell turnover, as increased apoptosis has been observed upon IL-18 treatment [145]. Also a redistribution from the blood to other tissues could be an underlying reason, even though this is less likely given the findings of persistent loss of MAIT cells also in the liver [144]. Thus, future research examining these mechanisms is warranted. Notably, also the implications of this persistent loss of MAIT cells are still uncertain. It can be hypothesized that this comes along with a weakened defense against pathogens, but also here further studies are wanted.

4.1.3 NK cells in chronic HCV

As described above, NK cells are thought to take part in controlling the HCV infection. Yet, once the chronic infection is established, NK cells are dysfunctional and display a skewed phenotype [138]. If one assesses NK cells not as one uniform population, but instead as different subpopulations with distinct functionalities, as for example the differences between $CD56^{bright}$ and $CD56^{dim}$ [41], the NK cell compartment becomes a diverse compartment with many subgroups [146]. Such NK cell diversity has been investigated in HIV infection, where in contrast to CD4 and CD8 T cells, only differences in NK cell diversity could be associated with chronic HIV infection [147]. In **Paper 2** we therefor investigated NK cell compartment and its diversity before, during, and after DAA-mediated clearance of HCV. In this study, the main cohort consisted of 26 patients with advanced fibrosis/cirrhosis that were treated with Sofosbuvir/Ribavirin and followed until week 36 after start of treatment; eight of these patients experienced a relapse after end of treatment **(Paper 2, Supplementary table 1**). Additionally, two cohorts with long term follow-up until week 120 were included for assessing long-term effect and influence of level of fibrosis: one cohort of nine patients with, and a second cohort of twelve patients without advanced fibrosis/cirrhosis at the start of treatment **(Paper 2, Supplementary table 2**).

4.1.4 Altered phenotype and NK cell diversity in HCV patients

To start with, we compared first the phenotype of $CD56^{bright}$ and $CD56^{dim}$ NK cell from HCV infected patients before treatment to healthy controls, both with SNE and single parameter analysis **(Paper 2, Figure 2** and **Supplementary Figure 1**). While many of the investigated markers remained unchanged, we could observe an effect on NK cells marked by lower expression of activation markers such as NKG2D and NKp46. SNE analysis also showed that these NK cells were in clusters with a similar pattern with lower levels of activation receptors that were more frequent in HCV patients, as highlighted by residual plots displaying the subtracted density plot of patients and controls.

Next, we validated our measures of both intra- and inter-individual NK cell diversity on a cohort of healthy donors stratified for CMV and Epstein-Barr-virus (EBV) infection as well as on NK cells from liver tissue before applying them on our HCV cohort **(Paper 2, Figure 3 and 4**). To this end, we calculated the diversity between individuals as standard deviation of the normalized marker expression (donor-to-donor expression variation, DEV) in relation to a previously reported cellular expression variation [148]; intraindividual NK cell diversity was calculated with the inverse Simpson diversity index (SDI) [146] based on differentiation subpopulations of $CD56^{bright}$ and $CD56^{dim}$ NK cells as well as expression of NKG2A, CD57 and KIR [62]. CMV infection leads in some individuals to persistent expansions of NK cell with a phenotype often marked by expression of NKG2C [67]. In line, independently of EBV serostatus, we observed in CMV positive individuals an increase in interindividual diversity for NKG2C and a significantly reduced SDI **(Paper 2, Figure 3**). Furthermore, intrahepatic NK cells, that exhibit in majority a $CD56^{bright}$ and CD16- phenotype [96], displayed reduced SDI but unaltered DEV.

After this validation step we next implemented these measures to compare healthy controls and chronic HCV patients (**Figure 4**). Indeed, both for both intra- and inter-individual diversity, significant alterations were observed in patients. The DEV was for almost all evaluated surface receptors elevated, whereas SDI was significantly decreased in chronic HCV infection. This means that while the NK cell compartment between patients was rather different, within each individual it showed a lower diversity. In contrast, acute HCV infection did not lead to such alterations within NK cells **(Paper 2, Supplementary Figure 2**), indicating that the chronic inflammation might be of importance.

Given the profound impact CMV infection can have on the NK cell compartment, we accordingly stratified phenotype, DEV, and SDI of HCV patients for CMV and other clinical parameters (sex, age, level of fibrosis and clinical outcome, **Paper 2, Figure 5,**

Supplementary Figure 3 and 4). Notably, none of these parameters displayed a relevant influence on NK cell phenotype, neither in PCA, SNE, or single parameter analysis. In line with this, neither CMV serostatus nor any other variable resulted in different levels of SDI. In contrast to this, only the degree of fibrosis led to significantly elevated DEV. We also investigated if altered diversity could be linked to the respective KIR and KIRL genotype, but only lower DEV appeared to be associated the KIRL/KIR combination C1/C1 KIR2DL3 **(Paper 2, Supplementary Figure 3**).

To assess whether the observed phenotypical alterations also had functional consequences, we subjected NK cells to stimulation with either K562 cells, 721.221 cells combined with Rituximab or IL-12/-18 stimulation **(Paper 2, Figure 6**). Here, we could not detect measurable differences when compared to healthy controls, neither in single cytokine expression nor in multifunctionality of NK cells. In contrast, NK cells from patients with milder liver disease (LDV/SOF) displayed somewhat enhanced functionality **(Paper 2, Supplementary Figure 5**). Furthermore, in patients with low intra-individual diversity previously reported defects in cytokine production [149] could be observed **(Paper 2, Supplementary Figure 2 and 5**). Altogether, we could not entirely recapitulate previous reported alterations in multifunctionality and degranulation [138,149,150]. For this either different patient characteristics might be responsible (for example the presence of advanced liver fibrosis in the majority of the here included patients) or also differing methodological approaches that might yield varying results (IL-12/-IL-21 [149] or IL-12/-15 treatment [150] versus the here used IL-12/-18 stimulation).

4.1.5 The NK cell compartment during and after viral clearance

Having shown that NK cell diversity is impacted by chronic infection with HCV, we next set out to investigate this in the context of rapid viral clearance **(Paper 2, Figure 7**). Remarkably, despite the rapid suppression of HCV, only discrete changes in the NK cell phenotype could be noted. Both expression levels and frequencies of most markers underwent only slight or no changes which could also be visualized by SNE analysis of the comparison between baseline and follow-up samples. Along the same lines, also NK cell diversity remained stable in the week 36 follow-up. Next, we assessed if restoration could be noted at the long-term follow-up in the additional two cohorts. Here, we could observe a partial restoration of the phenotype, as we noted a normalization of for example NKG2D and granzyme B up until week 120. In addition, we noted a reduction of DEV during the study period that we did not observe in the original cohort. This was contrasted by the SDI of the NK cell compartment, that did not reverse in the long-term follow-up and remained on the

comparable level. Furthermore, the function of NK cells throughout and after treatment appeared to be altered mainly during therapy **(Paper 2, Figure 8**) and here, mainly cytokine production of CD56dim NK cells was modulated.

To conclude, we were able to show an impact of the chronic infection with HCV on different levels of the NK cell compartment and its diversity. Of interest, there is conflicting literature on the observed alterations of NK cells in HCV patients, as for example NKG2D has been reported decreased [121], unaltered [150,151]and also elevated [149]. Some of these differences can be explained by either clinical inclusion criteria or flow cytometry analysis. Yet, we here show a higher inter-individual diversity in HCV patients indicating that HCV is able to drive not one specific but rather varying phenotypes. The NK cell population displayed remarkable stability, despite rapid elimination of HCV. The observed reduction in DEV in the long-term cohort might be associated to an improvement of liver function [152], given that in patients with more advanced liver disease a higher DEV was noted. In contrast, SDI and phenotype remained largely unchanged. Epigenetic modifications of NK cells have been reported in CMV infections [70], thus similar mechanism could also be responsible for the long-term effects we observed. Yet it has to be noted, that other studies could show a certain restoration of NK cell phenotype and function after DAA treatment[150,153]. However, these studies had no or only short follow-up after treatment, whereas we investigated NK cells from patients up until week 120 after viral clearance. Therefor it could be that some of the reported effects are only of short duration mediated by the treatment itself. On the other hand, also in our setting NK cell restoration might occur at even later time-points.

4.1.6 Conclusions and outlook

The availability of highly potent drugs against HCV revolutionized the treatment of this previously hard-to-treat chronic viral disease. For studying the underlying immunology of chronic viral infections and the immune system's role in viral control, this setting of rapid viral clearance is of great interest. Accordingly, diverse immune cells and also the cytokine profile have been studied in recent years before and after cure from HCV. The overall image to this day is that some functions and properties of the immune system appear to normalize, whereas others do not, as summarized in **Figure 1**. The imprint by chronic HCV can be exemplified by the study of Hengst *et al.* on soluble immune factors [154]. Here, the cytokine and chemokine profile of HCV patients was investigated in the context of DAA treatment and HCV cure. In brief, it could be shown that some, but not all soluble markers normalize and

others show only partial normalization. Further studies reported similar patterns of partial restoration of the soluble immune compartment [155,156]. Along the same lines, in CD8 T cells some reversal of dysfunction after viral clearance has been described [140] but also continued metabolic impairment [157] and persistence of HCV-specific PD-1+ CD127+ T cells[158] was reported. In γδT cells, an altered CD38 expression was noted before treatment in some patients [159], but also a stable γδT cell TCR repertoire until follow-up has been described[160]. We and others reported a persistent loss and dysfunction of MAIT cells[143,144,161], and lastly unchanged frequencies of T_{reg} after successful treatment have been described[162]. Thus, it might be that also the differing results regarding the NK cell compartment have to be seen in this light, that some NK cell functions and properties restore[150,153] but not all, as we report in **paper 2** [163]. One also has to have in mind, that despite viral clearance many of the patients are not immediately to be regarded as healthy: many might still have severe liver disease as consequence of the long-lasting infection, that only partially and slowly recovers [152]. This then leads to the question of how the persistent imprints on the immune system will influence the now cured patient's health. In regard of the high risk of complications from advanced fibrosis and cirrhosis, this should be investigated in future studies.

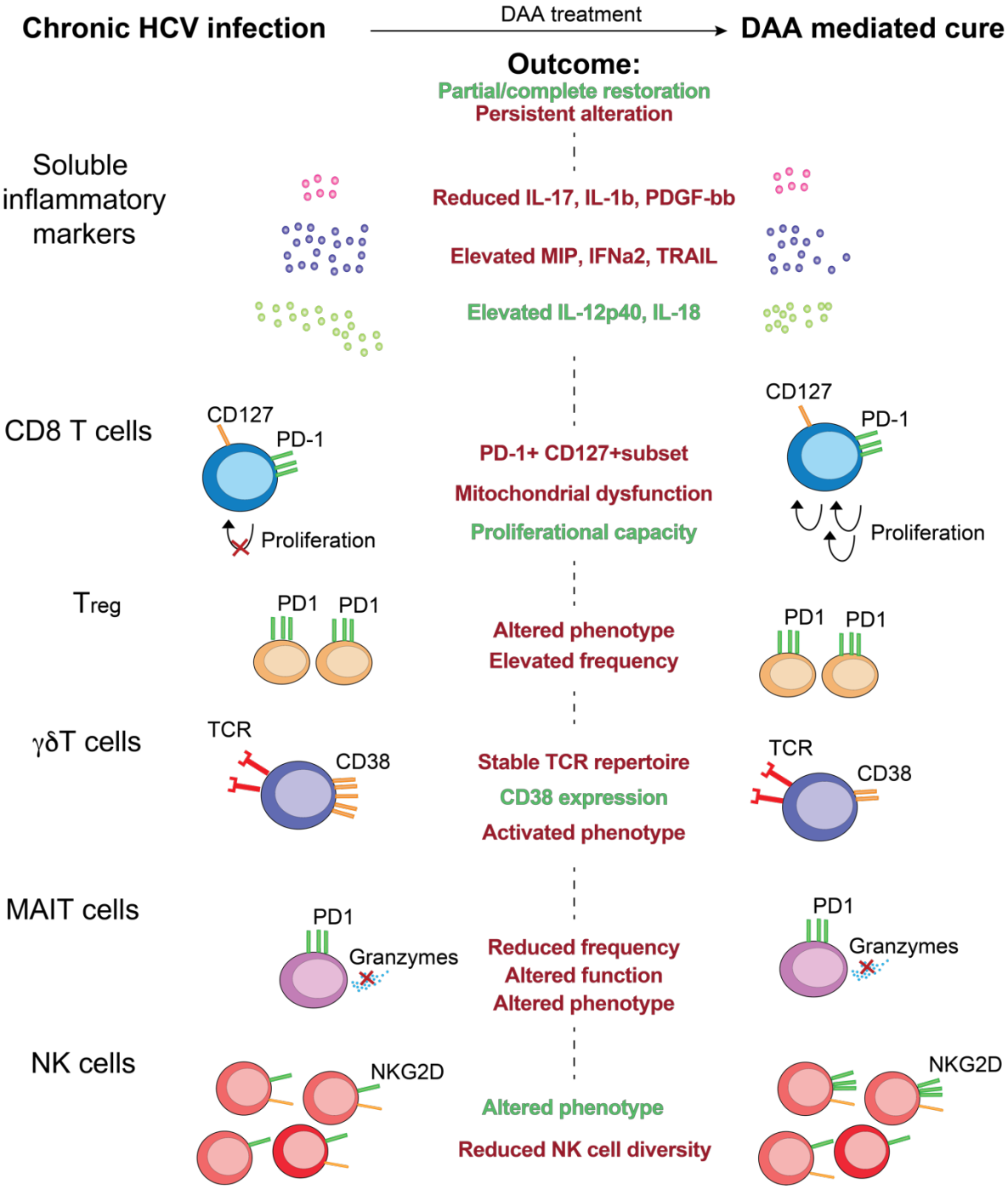

Figure 1. Changes in the immune system after rapid DAA mediated HCV cure. Colour-coded in middle row are the outcomes of the respective alteration after treatment (red = persistent alteration, green = normalization).

4.2 CIRRHOSIS, ASCITES AND LOCAL IMMUNE CELLS

In the case of liver cirrhosis and hepatic dysfunction, ascites is a common clinical presentation meaning accumulation of fluid in the peritoneal cavity. This is not only a cause of discomfort for patients, but also a threat as bacterial entry in ascites can lead to severe and life-threatening infections (SBP)[131]. In **paper 3** we therefor investigated the immune system

in ascites, with a focus on MAIT cells given their abundance in the liver [27] and their capacity to recognize bacterial metabolites [22]. To this end, healthy controls, patients with compensated and decompensated cirrhosis were included, as well as patients with diagnosed SBP. The underlying etiology for cirrhosis in these patients was either viral hepatitis (both HCV and HBV/HDV, abbreviated as HXV) or alcohol related cirrhosis (ARC). From patients with decompensated cirrhosis, matched ascites and peripheral blood samples were analyzed with flow cytometry and multiplexing of cytokines, from compensated cirrhosis and controls only peripheral blood samples.

4.2.1 Reduced frequency and activated phenotype of MAIT cells in cirrhotic patients

As a starting point we first investigated MAIT cells in peripheral blood of cirrhotic patients **(Paper 3, figure 1**). In line with previous reports [164], we observed reduced frequencies and an activated phenotype of MAIT cells in patients, indicated by elevated expression of HLA-DR, Ki-67, CD25, and CD38. However, we could not observe an altered function between patients and controls. When stratifying for complications (compensated/decompensated cirrhosis) and etiology, some differences could be observed, such as higher PD-1 expression in HXV- than ARC-related cirrhosis, whereas other alterations, such as elevated HLA-DR expression were common among all patients **(Paper 3, Supplementary figure 2**). Furthermore, we could detect higher levels of markers for monocyte or macrophage activation and indirect signs of bacterial translocation with elevated soluble CD14 (sCD14) and soluble CD163 (sCD163) in patients with liver cirrhosis [145,165,166]. When testing for a relation between liver disease, signs for bacterial translocation, and MAIT cells, we noted an inverse correlation between the frequency of MAIT cells and the severity of liver disease (indicated by Fibroscan and MELD score), as well as between frequency of MAIT cells and levels of sCD14 and sCD163. Altogether, these data indicate that a more advanced liver disease might associate with a higher risk for bacterial translocation/monocyte activation due to portal hypertension [167] and subsequent activation of MAIT cells [168]. The loss of MAIT cells in periphery could then be attributed either to lower production, continuous consumption or recruitment to local sites. Therefor it was of interest to next study the immune compartment within ascites.

4.2.2 MAIT cells are enriched, activated and functional in ascites

To get a broader picture of the immune profile in ascites, we performed flow cytometry on major immune cell subsets as well as screening of cytokine levels **(Paper 3, Figure 2 and 3**). Of note, the most prominently enriched cell type were $CD14^{+}CD16^{+}$ monocytes which could

be seen both in frequency and UMAP analysis. Given their expression of CD163 as well as greater size and granularity, these cells most likely represent peritoneal macrophages, that also under physiological conditions reside in the peritoneal cavity [169]. Looking at MAIT cells, also these were more frequent in ascites than in peripheral blood. Related to this, also within the cytokine milieu, a MAIT cell supportive composition was noted in ascites, with higher IL-7, a key cytokine for MAIT cell survival and functionality [21,170], as well as lower IL-18, able to activate but also induce apoptosis of MAIT cells [145].

Studying the phenotype of peritoneal MAIT cells in more detail, we could observe an enrichment of mainly CD8+ MAIT cells within the ascites **(Paper 3, Figure 3**). Furthermore, UMAP and Phenograph analysis revealed specific clusters that were more frequently present in ascites than in blood **(Paper 3, Figure 3 and 4**). These were marked by expression of the tissue residency markers CD69, CD103, and CD49a [171]. This suggests that tissue resident MAIT cells from the liver or intestine either get actively recruited to the ascites or leave these tissues due to increased venous and interstitial pressure in the portal drainage area. Interestingly, we could also observe higher levels of CXCR3 and CXCR4 on peritoneal MAIT cells **(Paper 3, Supplementary figure 4**), which in combination with elevated CXCL10 and peritoneal mesothelial cell derived CXCL12 [172], respectively, could lead to increased transition of MAIT cells from blood to ascites.

Next, we analyzed peritoneal MAIT cells in relation to the etiology of cirrhosis. Here, we noted minor phenotypic differences and, interestingly, a significantly reduced frequency of peritoneal MAIT cells in HXV patients compared to ARC, a pattern that was not seen in peripheral blood **(Paper 3, Figure 3** and **Supplementary figure 2**). It has been reported that the intrahepatic loss of MAIT cells in HCV is correlated to the degree of liver fibrosis and that the reduced abundance persists even after initiated DAA treatment [144]. Yet, the here studied cohorts of HXV and ARC patients displayed a comparable degree of liver disease. This might indicate that tissue-resident MAIT cells are reduced to a greater extent in HXV than in ARC, resulting in fewer MAIT cells that can be recruited to the ascites. However, further studies are needed to address this question in detail.

Finally, we studied how the functional capacities of peritoneal MAIT cells were influenced by the local milieu **(Paper 3, Figure 4**). Interestingly, both upon *E. coli* stimulation as well as cytokine priming, peritoneal MAIT cells presented with elevated functionality, seen as higher levels of IFNγ and granzyme B produced in response to both stimulations and TNF upon TCR-dependent stimulation. We also noted an inverse correlation between IL-18 levels in ascites and MAIT cell functionality. While IL-18 can activate MAIT cells, it might be that a

chronic stimulation leads to a certain dysfunction and apoptosis [145]. Given the abundance of peritoneal macrophages in ascites, we also tested whether these would influence local MAIT cell function but could not detect significant differences **(Paper 3, Supplementary figure 6**).

4.2.3 Enrichment of MAIT cells in spontaneous bacterial peritonitis

Given the increased abundance of MAIT cells and ascites and their ability to respond to bacterial infection, it was of interest to study peritoneal MAIT cells in SBP **(Paper 3, Figure 5**). Notably, in this condition, MAIT cells were even further enriched in ascites, both in relative abundance and in absolute numbers. Moreover, when looking at all immune cells comparing SBP to non-SBP, MAIT cells were the most enriched immune cell subset. Next, we investigated if differences could be noted in their phenotype and in the cytokine milieu. Yet, both regarding MAIT cell phenotype and the cytokine profile we observed surprisingly few differences between SBP and non-SBP. One of the cytokines that was most strongly reduced was IL-7, which appears to be in contrast to the elevated MAIT cell frequency. On the other hand, CCL5 was increased in ascites from SBP patients and can bind to CCR5, which is highly expressed on circulating MAIT cells [24]. As also tissue resident phenotypes were slightly more prevalent in non-SBP patients, this could indicate an enhanced recruitment of MAIT cells to ascites from circulation during SBP.

4.2.4 Summary and future studies

Liver disease is frequent and cirrhosis a severe condition with strongly increased mortality [127]. A frequent complication of cirrhosis is ascites and connected to this also SBP [130,131]. Here we performed a comprehensive analysis of the immune system located to ascites of patients with or without SBP. We could show an enrichment of MAIT cells in the ascites, that these cells display an activated phenotype, and that they are highly functional. This is of interest, as in many other chronic conditions, MAIT cell frequency and functionality was found to be reduced [31,34,161]. One explanation for the elevated frequency and function of peritoneal MAIT cells might be the local cytokine milieu that is supporting MAIT cells, as for example IL-7 was present at higher levels. Additionally, peritoneal MAIT cells appeared to be recruited to some extent from tissue, as shown by the elevated expression of tissue residency markers. This could be mediated by the chemokine receptor expression on MAIT cells (high levels of CXCR3 and CXCR4) and the cytokines present in the peritoneal cavity (CXCL10 and CXCL12) [172]. Given their ability to respond readily with innate effector functions, MAIT cells present an interesting target for possible interventions and treatments in bacterial infections [173]. Thus, future studies should evaluate if and how MAIT cell modulation is possible in these conditions. Also, an outstanding question for future investigations is if the

loss of MAIT cells in cirrhosis in peripheral blood already might predispose to bacterial infection.

4.3 TISSUE RESIDENT NK CELLS IN THE UTERUS

The uterus has a unique composition of immune cells. In contrast to peripheral blood, the relative abundance of residing immune cells is shifted away from T cells towards NK cells and monocytes/macrophages [64,174]. The underlying theory is that this shift together with other mechanisms, as for example higher levels of local T_{reg} [175], aims to prevent T cell allorecognition of the fetus. In this context it is of interest, that especially NK cells are found at higher frequency and with a unique phenotype of co-expression of NKG2A and KIR on $CD56^{bright}$ NK cells, that is not present in peripheral blood [84]. Recent advances in single-cell transcriptomics revealed even an even greater heterogeneity with the existence of a subgroup of NK cells expressing CD39 [176]. This is of interest, as CD39 is known to be expressed by T_{reg} and that it mediates immune suppressive effects via cleavage of extracellular ATP [177]. Thus, it is intriguing if uNK cells possibly could have such a function, too. Yet, it remains unclear how this immune composition and NK cell phenotype can be upheld in regard to the continuously regenerating endometrium that is shed each month during menstruation. To investigate this, we performed **study 4**, and assessed uterine NK cell differentiation and function in the regenerating endometrium and in pregnancy.

4.3.1 Uterine NK cells are distinct in blood, endometrium and decidua and comprise three main subsets

As a starting point, we set out to determine the global phenotypic profile of uNK cells in endometrium, decidua, and peripheral blood **(Paper 4, Figure 1**). To this end we barcoded cells from three donors from each tissue and screened the surface proteome with the LegendScreen (Biolegend). We validated our data by detecting previously reported markers of uNK cells that are expressed both in endometrium and decidua, such as KIR, CD9, and CD49a [84,86]. Notably, we also uncovered novel molecules uniformly expressed on uNK cells, among others GITR, CD82, and Sialyl Lewis X. Additionally we could discriminate patterns more prevalent in either decidua or endometrium, as seen in hierarchical clustering, PCA, and single parameter analysis. Here, activating receptors (such as NKG2D, NKp46 and NKp30) were expressed more frequently on decidual uNK cells whereas cytokine and chemokine receptors where elevated on endometrial uNK cells (IL-21R, CXCR7).

Having outlined the global pattern and differences, we then investigated it in more detail in the context of NK cell differentiation **(Paper 4, Figure 2**). The acquisition of KIRs is tightly

linked to differentiation of peripheral blood NK cells [62]. Therefore, we investigated the surface proteome in KIR- and KIR+ uNK cells and could identify distinct patterns, among which we also detected the previously reported CD39 nearly exclusively expressed on KIR+ uNK cells. Further stratification to the three distinct subsets of KIR-CD39-, KIR+CD39- and KIR+CD39+ uNK cells suggested a step-wise model of receptor acquisition. In addition, we could observe both higher expression levels of KIRs and more KIRs simultaneously co-expressed per cell. Together, these data suggest that, equivalent to peripheral blood differentiation, uNK cells differentiate from KIR-CD39- over KIR+CD39- to KIR+CD39+ cells. Moreover, we could underline these findings with additional results. Data from RNA sequencing of the three subsets and from CyTOF analysis confirmed that uNK cells aligned along this hypothesized differentiational pathway in PCA and Wishbone trajectory analysis, respectively. Finally, we also re-analyzed published single-cell RNAseq data [176]with Pseudotime analysis (SlingShot), a method used for displaying continuous changes (as in differentiation) in single cell transcriptomic data. Also in this analysis, we could recapitulate a subsequent acquisition of KIR and finally CD39, that was accompanied by altered marker expression. Linking this data together, we found consistent phenotypic alterations across several of our datasets including loss of CD27, CD11c and CXCR4, and upregulation of KIR, CD39, and LILRB1.

4.3.2 uNK cell differentiation is coupled to endometrial regeneration and pregnancy

After having defined a possible differentiational axis of uNK cells, we then set out to analyze this pathway throughout the menstrual cycle. To this end, we collected samples from ten healthy women during the proliferative and secretory phase (using endometrial biopsies), as well as menstrual blood (representing the end of the cycle). First, we assessed the immune cell distribution and the respective subset proliferation throughout the cycle. Strikingly, uNK cells peaked in proliferation during the later phase of the cycle. When analyzing the outlined uNK cell subsets throughout the cycle as well as in early pregnancy (decidua) and in postmenopause (PMP), we saw an increase of the relative abundance of KIR+CD39+ uNK cells in menstrual blood and thus after the peak in proliferation. This level was then also seen in pregnancy, whereas nearly no NK cells with a uNK cell phenotype could be observed in PMP. This indicates that sex hormones play a crucial role in shaping the endometrial immune compartment and that it is continuously changing.

4.3.3 The regulation and tissue residency of KIR+CD39+ uNK cells.

In the analyses this far, we noted a pronounced variation in the relative abundance of KIR+CD39+ uNK cells. It has been shown, that CD39 is one of the molecules with a high genetic inheritance on T_{reg} [178]. Therefor we set out to study the frequency of uNK cell subsets in monozygotic twins. Here we noted a high concordance among each twin pair, indicating that also on uNK cells a genetic component might be of importance. Yet, as the endometrium is shed each month, we were intrigued to study further if and how this tissue resident population could be generated. There have been studies supporting the two concepts of either local repopulation or recruitment from periphery. On the one hand, resident CD34+ progenitors have been described that might give rise to a local population [179], as well as data from parabiosis experiments in mice, showing a tissue resident uNK cell population [180]. On the other hand, an inducible uNK cell phenotype [181]and recruitment from peripheral blood was suggested [182]. We had the chance to study endometrial samples from HLA-mismatched uterus transplanted patients to investigate the origin of uNK cells according to their HLA-type. Indeed, in the cases investigated we could identify *bona fidae* uNK cells that appeared to be from recipient origin, suggesting a recruitment from periphery.

4.3.4 IL-15 drives uNK cell differentiation in vitro and in vivo

Given the observed dynamics of uNK cell subsets throughout the menstrual cycle, a likely link between endometrial regeneration and uNK cell differentiation is the previously described progesterone-mediated production of IL-15 by stromal cells [183,184]. Therefor we tested the differentiation and proliferation of sorted uNK cell subsets both *in vitro* and *in vivo* **(Paper 4, Figure 4**). Indeed, resembling the pattern of peripheral blood NK cell differentiation [62], the ability to proliferate was continuously lost from KIR-CD39- over KIR+CD39- to KIR+CD39+ uNK cells. Furthermore, we noted upregulation of CD39 almost exclusively on proliferating cells. Similarly, after injection of sorted uNK cells in MISTRG mice [185,186], upregulation of KIR and CD39 or only CD39 was seen on KIR-CD39- or KIR+CD39- uNK cells, respectively. Altogether, this would suggest that uNK cells differentiate in response to IL-15.

4.3.5 Differentiation of uNK cells is linked to altered functionality and reduced inflammatory pattern

A hallmark of lymphocyte differentiation is that it is coupled to modified functionality. Therefor we next studied possible alterations also during uNK cell differentiation. As uNK cells are known to be weakly cytotoxic [64], we first aimed to investigate the profile of released soluble factors by the respective subsets. To this end, we analyzed supernatants after

stimulation with PEA and measured 92 soluble markers **(Paper 4, Figure 5**). Indeed, we observed a shift from more pro-inflammatory cytokines, such as IFNγ, CCL4, and TNF produced by KIR$^-$CD39$^-$ uNK cells, towards more immuno-modulatory factors like galectin-1 and -9 more highly produced by KIR+CD39+ uNK cells. The latter factors have been proposed to be involved in modulating T cell immunity [187,188], and thus suggesting an immune-modulatory role of KIR+CD39+ uNK cells. Looking at the differential profile of immune factor production, we noted, in line with the observations in the surface proteome, that the KIR+CD39- subsets appeared to represent an intermediate state between KIR-CD39- and KIR+CD39+.

We then went on to confirm these findings with flow cytometry. As expected, we could observe the distinct reduction of pro-inflammatory cytokines in KIR+CD39+ uNK cells. Of note, these cells were able to degranulate more and had higher levels of perforin, which is in line with previous work showing higher granule content in KIR-expressing uNK cells [189]. Concluding, we noted a skewing of uNK cell functionality away from pro-inflammatory markers to possibly immune modulating markers.

4.3.6 Conclusion and perspectives

Despite advances in research, many issues in the immunology of the uterus and pregnancy remain unsolved. Here, we aimed to address the question of how the locally residing immune cells respond to and keep up with the repetitive regeneration of the human endometrium and its transformation to decidual tissue. We present a possible pathway of differentiation originating from KIR-CD39- uNK cells over acquisition of first KIR and then CD39 to KIR+CD39+ cells. Furthermore, we could link this differentiation to the menstrual cycle and propose progesterone induced IL-15 as main driver of this process [184,190]. Along this proposed differentiational axis we noted a shift in uNK cell function away pro-inflammatory cytokines that were mainly produced by KIR$^-$CD39$^-$ uNK cells, towards more immuno-modulatory, such as galectin-1 and -9 [187,188]. The latter have also been associated with angiogenic properties [191]. Thus, it might be plausible that the late rise of KIR+CD39+ uNK cells is of importance especially during the early phase of pregnancy, given their late appearance in the cycle and their functional properties. Furthermore, CD39 itself might contribute locally to a more immunosuppressive environment, especially when in contact with CD73-expressing trophoblast [176]. The two ectoenzymes CD39 and CD73 work in concert to cleave extracellular ATP into adenosine, which in turn has immunosuppressive effects [192]. Hence, the combination of skewed functionality and increased levels of extracellular adenosine might contribute to the local immunological milieu needed for the establishment of a pregnancy.

However, we noted a significant variation among individuals with KIR+CD39+ uNK cells representing all from 4% to 64% of the uNK cell population. As these cells express high levels of KIR and as associations between KIR/KIRL genotypes and pregnancy associated pathologies have been reported [87,88], this variation is of great interest and should be investigated in future studies in relation to the clinical outcome.

5 CONCLUDING REMARKS

The immune system is involved in the control of infections and tissue function. To this end, MAIT cells and NK cells are present at different sites and display at each a particular phenotype and functionality. In chronic infections, these can be severely altered but little is known if and how imprints remain after clearance of the chronic insult. Here, we studied MAIT and NK cell function in different settings of tissue homeostasis, tissue pathology as well as during and after cure of a chronic viral infection. Below, key findings of each study are listed:

- MAIT cell frequency is reduced in chronic HCV patients and the remaining cells are dysfunctional **(Paper 1**)
- The altered frequency, phenotype, and function of MAIT cells persist after rapid clearance of chronic HCV infection **(Paper 1**)
- In patients with chronic HCV, NK cell phenotype and diversity are altered **(Paper 2**)
- Only partial restoration of the NK cell phenotype occurs upon rapid HCV cure **(Paper 2**)
- Intraindividual NK cell diversity (SDI) is not altered after clearance of HCV, whereas interindividual diversity (DEV) improves in association with amelioration of liver disease **(Paper 2**)
- MAIT cells are more frequent, express tissue residency markers and display elevated functionality in the ascites of cirrhotic patients **(Paper 3**)
- Upon SBP, peritoneal MAIT cells are further enriched compared to non-SBP patients **(Paper 3**)
- uNK cells display a specific surface proteome distinct from peripheral blood **(Paper 4**)
- Differentiation of uNK cells is linked to the menstrual cycle and KIR+CD39+ uNK cells arise late in the cycle and in pregnancy **(Paper 4**)
- Functional skewing of KIR+CD39+ uNK cells away from pro-inflammatory cytokines towards degranulation and immuno-modulatory and angiogenic factors **(Paper 4**)

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7 REFERENCES

1. Parkin, J. & Cohen, B. An overview of the immune system. *Lancet* **357,** 1777–1789 (2001).
2. Shortman, K. & Liu, Y.-J. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* **2,** 151–161 (2002).
3. Love, P. E. & Bhandoola, A. Signal integration and crosstalk during thymocyte migration and emigration. *Nat. Rev. Immunol.* **11,** 469–477 (2011).
4. Clark, M. R., Mandal, M., Ochiai, K. & Singh, H. Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. *Nat. Rev. Immunol.* **14,** 69–80 (2014).
5. Shapiro-Shelef, M. & Calame, K. Regulation of plasma-cell development. *Nat. Rev. Immunol.* **5,** 230–242 (2005).
6. Neefjes, J., Jongsma, M. L. M., Paul, P. & Bakke, O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat. Rev. Immunol.* **11,** 823–836 (2011).
7. Joffre, O. P., Segura, E., Savina, A. & Amigorena, S. Cross-presentation by dendritic cells. *Nat. Rev. Immunol.* **12,** 557–569 (2012).
8. Alt, F. W. *et al.* VDJ recombination. *Immunol. Today* **13,** 306–314 (1992).
9. Godfrey, D. I., Uldrich, A. P., McCluskey, J., Rossjohn, J. & Moody, D. B. The burgeoning family of unconventional T cells. *Nat. Immunol.* **16,** 1114–1123 (2015).
10. Seder, R. A. & Ahmed, R. Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. *Nat. Immunol.* **4,** 835–842 (2003).
11. Byrne, J. A., Butler, J. L. & Cooper, M. D. Differential activation requirements for virgin and memory T cells. *J. Immunol.* **141,** 3249–3257 (1988).
12. Jameson, S. C. & Masopust, D. Diversity in T cell memory: an embarrassment of riches. *Immunity* **31,** 859–871 (2009).
13. Sallusto, F., Lenig, D., Förster, R., Lipp, M. & Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **401,** 708–712 (1999).
14. Vignali, D. A. A., Collison, L. W. & Workman, C. J. How regulatory T cells work. *Nat. Rev. Immunol.* **8,** 523–532 (2008).
15. King, C. New insights into the differentiation and function of T follicular helper cells. *Nat. Rev. Immunol.* **9,** 757–766 (2009).
16. Kinjo, Y. *et al.* Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat. Immunol.* **7,** 978–986 (2006).
17. Bukowski, J. F., Morita, C. T. & BRENNER, M. B. Human gamma delta T cells recognize alkylamines derived from microbes, edible plants, and tea: Implications for innate immunity. *Immunity* **11,** 57–65 (1999).
18. Das, H. *et al.* MICA engagement by human V gamma 2V delta 2 T cells enhances their antigen-dependent effector function. *Immunity* **15,** 83–93 (2001).
19. Kong, Y. *et al.* The NKG2D ligand ULBP4 binds to TCRgamma9/delta2 and induces cytotoxicity to tumor cells through both TCRgammadelta and NKG2D. *Blood* **114,** 310–317 (2009).
20. Treiner, E. *et al.* Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* **422,** 164–169 (2003).

21. Leeansyah, E. *et al.* Arming of MAIT Cell Cytolytic Antimicrobial Activity Is Induced by IL-7 and Defective in HIV-1 Infection. *PLoS Pathog.* **11,** e1005072 (2015).
22. Kjer-Nielsen, L. *et al.* MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491,** 717–723 (2012).
23. Le Bourhis, L. *et al.* MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. *PLoS Pathog.* **9,** e1003681 (2013).
24. Dias, J., Leeansyah, E. & Sandberg, J. K. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci USA* **114,** E5434–E5443 (2017).
25. Ussher, J. E. *et al.* CD161++CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *Eur. J. Immunol.* **44,** 195–203 (2014).
26. Tang, X.-Z. *et al.* IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *The Journal of Immunology* **190,** 3142–3152 (2013).
27. Dusseaux, M. *et al.* Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* **117,** 1250–1259 (2011).
28. Lanier, L. L., Chang, C. & Phillips, J. H. Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *The Journal of Immunology* **153,** 2417–2428 (1994).
29. Fergusson, J. R. *et al.* CD161 defines a transcriptional and functional phenotype across distinct human T cell lineages. *Cell Rep* **9,** 1075–1088 (2014).
30. Leeansyah, E., Loh, L., Nixon, D. F. & Sandberg, J. K. Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nat Commun* **5,** 3143 (2014).
31. Leeansyah, E. *et al.* Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. *Blood* **121,** 1124–1135 (2013).
32. Kwon, Y.-S. *et al.* Mucosal-associated invariant T cells are numerically and functionally deficient in patients with mycobacterial infection and reflect disease activity. *Tuberculosis (Edinb)* **95,** 267–274 (2015).
33. Magalhaes, I. *et al.* Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. *J. Clin. Invest.* **125,** 1752–1762 (2015).
34. Cho, Y.-N. *et al.* Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. *J. Immunol.* **193,** 3891–3901 (2014).
35. Sandberg, J. K., Dias, J., Shacklett, B. L. & Leeansyah, E. Will loss of your MAITs weaken your HAART [corrected]? *AIDS* **27,** 2501–2504 (2013).
36. Loh, L. *et al.* Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation. *Proc Natl Acad Sci U S A* **113,** 10133–10138 (2016).
37. van Wilgenburg, B. *et al.* MAIT cells contribute to protection against lethal influenza infection in vivo. *Nat Commun* **9,** 4706–9 (2018).
38. van Wilgenburg, B. *et al.* MAIT cells are activated during human viral infections. *Nat Commun* **7,** 11653 (2016).
39. Cosgrove, C. *et al.* Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. *Blood* **121,** 951–961 (2013).
40. Gordon, S. Pattern recognition receptors: doubling up for the innate immune response. *Cell* **111,** 927–930 (2002).
41. Vivier, E., Tomasello, E., Baratin, M., Walzer, T. & Ugolini, S. Functions of natural killer cells. *Nat. Immunol.* **9,** 503–510 (2008).

42. Gunesch, J. T. *et al.* CD56 regulates human NK cell cytotoxicity through Pyk2. *Elife* **9,** (2020).
43. Lanier, L. L. Up on the tightrope: natural killer cell activation and inhibition. *Nat. Immunol.* **9,** 495–502 (2008).
44. Enqvist, M. *et al.* Coordinated expression of DNAM-1 and LFA-1 in educated NK cells. *J. Immunol.* **194,** 4518–4527 (2015).
45. Ljunggren, H. G. & Kärre, K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* **11,** 237–244 (1990).
46. Lanier, L. L., Corliss, B. C., Wu, J., Leong, C. & Phillips, J. H. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* **391,** 703–707 (1998).
47. Colonna, M. & Samaridis, J. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. *Science* **268,** 405–408 (1995).
48. Boyington, J. C., Motyka, S. A., Schuck, P., Brooks, A. G. & Sun, P. D. Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand. *Nature* **405,** 537–543 (2000).
49. Uhrberg, M. *et al.* Human diversity in killer cell inhibitory receptor genes. *Immunity* **7,** 753–763 (1997).
50. Parham, P. Killer cell immunoglobulin-like receptor diversity: balancing signals in the natural killer cell response. *Immunol. Lett.* **92,** 11–13 (2004).
51. Hsu, K. C., Chida, S., Geraghty, D. E. & Dupont, B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. *Immunol. Rev.* **190,** 40–52 (2002).
52. Parham, P. MHC class I molecules and KIRs in human history, health and survival. *Nat. Rev. Immunol.* **5,** 201–214 (2005).
53. Manser, A. R., Weinhold, S. & Uhrberg, M. Human KIR repertoires: shaped by genetic diversity and evolution. *Immunol. Rev.* **267,** 178–196 (2015).
54. Stern, M. *et al.* Telomeric rather than centromeric activating KIR genes protect from cytomegalovirus infection after kidney transplantation. *Am. J. Transplant.* **11,** 1302–1307 (2011).
55. Nakimuli, A. *et al.* Allele-level haplotype frequencies and pairwise linkage disequilibrium for 14 KIR loci in 506 European-American individuals. *Proc Natl Acad Sci USA* **112,** 845–850 (2015).
56. Parham, P. & Moffett, A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat. Rev. Immunol.* **13,** 133–144 (2013).
57. Khakoo, S. I. *et al.* HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* **305,** 872–874 (2004).
58. Kulkarni, S., Martin, M. P. & Carrington, M. The Yin and Yang of HLA and KIR in human disease. *Semin. Immunol.* **20,** 343–352 (2008).
59. Kim, S. *et al.* Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* **436,** 709–713 (2005).
60. Anfossi, N. *et al.* Human NK cell education by inhibitory receptors for MHC class I. *Immunity* **25,** 331–342 (2006).
61. Kim, S. *et al.* HLA alleles determine differences in human natural killer cell responsiveness and potency. *Proc Natl Acad Sci USA* **105,** 3053–3058 (2008).
62. Björkström, N. K. *et al.* Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. *Blood* **116,** 3853–3864 (2010).

63. Björkström, N. K., Ljunggren, H.-G. & Michaëlsson, J. Emerging insights into natural killer cells in human peripheral tissues. *Nat. Rev. Immunol.* **16,** 310–320 (2016).
64. Moffett, A. & Colucci, F. Uterine NK cells: active regulators at the maternal-fetal interface. *J. Clin. Invest.* **124,** 1872–1879 (2014).
65. Sun, J. C., Beilke, J. N. & Lanier, L. L. Adaptive immune features of natural killer cells. *Nature* **457,** 557–561 (2009).
66. Cook, M. *et al.* Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation. *Blood* **107,** 1230–1232 (2006).
67. Béziat, V. *et al.* NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. *Blood* **121,** 2678–2688 (2013).
68. Guma, M. *et al.* Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* **104,** 3664–3671 (2004).
69. Hammer, Q. *et al.* Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nat. Immunol.* 1–20 (2018). doi:10.1038/s41590-018-0082-6
70. Schlums, H. *et al.* Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity* **42,** 443–456 (2015).
71. Fan, X. & Rudensky, A. Y. Hallmarks of Tissue-Resident Lymphocytes. *Cell* **164,** 1198–1211 (2016).
72. Kramer, R. H. & Marks, N. Identification of integrin collagen receptors on human melanoma cells. *J. Biol. Chem.* **264,** 4684–4688 (1989).
73. Cepek, K. L. *et al.* Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature* **372,** 190–193 (1994).
74. Shiow, L. R. *et al.* CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* **440,** 540–544 (2006).
75. Mackay, L. K. *et al.* Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. *J. Immunol.* **194,** 2059–2063 (2015).
76. Schenkel, J. M. & Masopust, D. Tissue-resident memory T cells. *Immunity* **41,** 886–897 (2014).
77. Red-Horse, K. *et al.* Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *Journal of Clinical Investigation* **114,** 744–754 (2004).
78. Harris, L. K. Review: Trophoblast-vascular cell interactions in early pregnancy: how to remodel a vessel. *Placenta* **31 Suppl,** S93–8 (2010).
79. Zenclussen, A. C. Adaptive immune responses during pregnancy. *Am. J. Reprod. Immunol.* **69,** 291–303 (2013).
80. Apps, R. *et al.* Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. *Immunology* **127,** 26–39 (2009).
81. Ramhorst, R. *et al.* Modulation and recruitment of inducible regulatory T cells by first trimester trophoblast cells. *Am. J. Reprod. Immunol.* **67,** 17–27 (2012).
82. Fraccaroli, L. *et al.* VIP boosts regulatory T cell induction by trophoblast cells in an in vitro model of trophoblast-maternal leukocyte interaction. *J. Leukoc. Biol.* **98,** 49–58 (2015).

83. Moffett, A. & Colucci, F. Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction. *Immunol. Rev.* **267,** 283–297 (2015).
84. Manaster, I. *et al.* Endometrial NK cells are special immature cells that await pregnancy. *J. Immunol.* **181,** 1869–1876 (2008).
85. Manaster, I. & Mandelboim, O. The unique properties of uterine NK cells. *Am. J. Reprod. Immunol.* **63,** 434–444 (2010).
86. Ivarsson, M. A. *et al.* Composition and dynamics of the uterine NK cell KIR repertoire in menstrual blood. *Mucosal Immunol* (2016). doi:10.1038/mi.2016.50
87. Hiby, S. E. *et al.* Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* **200,** 957–965 (2004).
88. Hiby, S. E. *et al.* Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. *The Journal of Immunology* **192,** 5069–5073 (2014).
89. Sharkey, A. M. *et al.* Tissue-Specific Education of Decidual NK Cells. *J. Immunol.* **195,** 3026–3032 (2015).
90. Vacca, P. *et al.* Analysis of natural killer cells isolated from human decidua: Evidence that 2B4 (CD244) functions as an inhibitory receptor and blocks NK-cell function. *Blood* **108,** 4078–4085 (2006).
91. Male, V. *et al.* The effect of pregnancy on the uterine NK cell KIR repertoire. *Eur. J. Immunol.* **41,** 3017–3027 (2011).
92. Kieckbusch, J., Gaynor, L. M., Moffett, A. & Colucci, F. MHC-dependent inhibition of uterine NK cells impedes fetal growth and decidual vascular remodelling. *Nat Commun* **5,** 3359 (2014).
93. Fu, B. *et al.* Natural Killer Cells Promote Fetal Development through the Secretion of Growth-Promoting Factors. *Immunity* **47,** 1100–1113.e6 (2017).
94. Robinson, M. W., Harmon, C. & O'Farrelly, C. Liver immunology and its role in inflammation and homeostasis. *Cell. Mol. Immunol.* **13,** 267–276 (2016).
95. Norris, S. *et al.* Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. *J. Hepatol.* **28,** 84–90 (1998).
96. Hudspeth, K. *et al.* Human liver-resident CD56(bright)/CD16(neg) NK cells are retained within hepatic sinusoids via the engagement of CCR5 and CXCR6 pathways. *J. Autoimmun.* **66,** 40–50 (2016).
97. Stegmann, K. A. *et al.* CXCR6 marks a novel subset of T-bet(lo)Eomes(hi) natural killer cells residing in human liver. *Nature Publishing Group* **6,** 26157–10 (2016).
98. Filipovic, I. *et al.* 29-Color Flow Cytometry: Unraveling Human Liver NK Cell Repertoire Diversity. *Front Immunol* **10,** 2692 (2019).
99. Aw Yeang, H. X. *et al.* Cutting Edge: Human CD49e- NK Cells Are Tissue Resident in the Liver. *J. Immunol.* **198,** 1417–1422 (2017).
100. MacParland, S. A. *et al.* Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat Commun* **9,** 4383–21 (2018).
101. Krenkel, O. & Tacke, F. Liver macrophages in tissue homeostasis and disease. *Nat. Rev. Immunol.* **17,** 306–321 (2017).
102. Gossard, A. A. & Lindor, K. D. Autoimmune hepatitis: a review. *J. Gastroenterol.* **47,** 498–503 (2012).
103. Greuter, T., Malhi, H., Gores, G. J. & Shah, V. H. Therapeutic opportunities for alcoholic steatohepatitis and nonalcoholic steatohepatitis: exploiting similarities and differences in pathogenesis. *JCI Insight* **2,** 434 (2017).
104. Pimpin, L. *et al.* Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. *J. Hepatol.* **69,** 718–735 (2018).
105. Rizzetto, M. Hepatitis D: thirty years after. *J. Hepatol.* **50,** 1043–1050 (2009).

106. Blachier, M., Leleu, H., Peck-Radosavljevic, M., Valla, D.-C. & Roudot-Thoraval, F. The burden of liver disease in Europe: a review of available epidemiological data. *J. Hepatol.* **58,** 593–608 (2013).
107. Rehermann, B. & Bertoletti, A. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. *Hepatology* **61,** 712–721 (2015).
108. Rehermann, B. & Nascimbeni, M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat. Rev. Immunol.* **5,** 215–229 (2005).
109. Ohto, H. *et al.* Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. *N. Engl. J. Med.* **330,** 744–750 (1994).
110. Grebely, J. & Dore, G. J. What is killing people with hepatitis C virus infection? *Semin. Liver Dis.* **31,** 331–339 (2011).
111. Alter, M. J. Epidemiology of hepatitis C. in **26,** 62S–65S (1997).
112. Hajarizadeh, B., Grebely, J. & Dore, G. J. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* **10,** 553–562 (2013).
113. Gower, E., Estes, C., Blach, S., Razavi-Shearer, K. & Razavi, H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J. Hepatol.* **61,** S45–57 (2014).
114. Kohli, A., Shaffer, A., Sherman, A. & Kottilil, S. Treatment of hepatitis C: a systematic review. *JAMA* **312,** 631–640 (2014).
115. Wedemeyer, H. *et al.* Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. *The Journal of Immunology* **169,** 3447–3458 (2002).
116. Spangenberg, H. C. *et al.* Intrahepatic CD8+ T-cell failure during chronic hepatitis C virus infection. *Hepatology* **42,** 828–837 (2005).
117. Urbani, S. *et al.* PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. *J. Virol.* **80,** 11398–11403 (2006).
118. Golden-Mason, L. *et al.* Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. *J. Virol.* **83,** 9122–9130 (2009).
119. Nakamoto, N. *et al.* Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog.* **5,** e1000313 (2009).
120. Shen, T. *et al.* PD-1 expression on peripheral CD8+ TEM/TEMRA subsets closely correlated with HCV viral load in chronic hepatitis C patients. *Virol. J.* **7,** 310 (2010).
121. Sène, D. *et al.* Hepatitis C virus (HCV) evades NKG2D-dependent NK cell responses through NS5A-mediated imbalance of inflammatory cytokines. *PLoS Pathog.* **6,** e1001184 (2010).
122. Yoon, J. C., Lim, J.-B., Park, J. H. & Lee, J. M. Cell-to-cell contact with hepatitis C virus-infected cells reduces functional capacity of natural killer cells. *J. Virol.* **85,** 12557–12569 (2011).
123. Dolganiuc, A., Paek, E., Kodys, K., Thomas, J. & Szabo, G. Myeloid dendritic cells of patients with chronic HCV infection induce proliferation of regulatory T lymphocytes. *Gastroenterology* **135,** 2119–2127 (2008).
124. Kanto, T. *et al.* Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. *J. Infect. Dis.* **190,** 1919–1926 (2004).
125. Pawlotsky, J.-M., Feld, J. J., Zeuzem, S. & Hoofnagle, J. H. From non-A, non-B hepatitis to hepatitis C virus cure. *J. Hepatol.* **62,** S87–99 (2015).

126. Tsochatzis, E. A., Bosch, J. & Burroughs, A. K. Liver cirrhosis. *Lancet* **383,** 1749–1761 (2014).
127. D'Amico, G., Garcia-Tsao, G. & Pagliaro, L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J. Hepatol.* **44,** 217–231 (2006).
128. Castera, L., Pinzani, M. & Bosch, J. Non invasive evaluation of portal hypertension using transient elastography. *J. Hepatol.* **56,** 696–703 (2012).
129. Albillos, A., Lario, M. & Álvarez-Mon, M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J. Hepatol.* **61,** 1385–1396 (2014).
130. Arvaniti, V. *et al.* Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* **139,** 1246–56– 1256.e1–5 (2010).
131. Fernández, J. *et al.* Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* **35,** 140–148 (2002).
132. Eden, E., Navon, R., Steinfeld, I., Lipson, D. & Yakhini, Z. GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* **10,** 48 (2009).
133. Liberzon, A. *et al.* Molecular signatures database (MSigDB) 3.0. *Bioinformatics* **27,** 1739–1740 (2011).
134. Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* **1,** 417–425 (2015).
135. Mootha, V. K. *et al.* PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34,** 267–273 (2003).
136. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* **102,** 15545–15550 (2005).
137. Alter, G. *et al.* Reduced frequencies of NKp30+NKp46+, CD161+, and NKG2D+ NK cells in acute HCV infection may predict viral clearance. *J. Hepatol.* **55,** 278–288 (2011).
138. Lunemann, S. *et al.* Compromised function of natural killer cells in acute and chronic viral hepatitis. *J. Infect. Dis.* **209,** 1362–1373 (2014).
139. Alanio, C. *et al.* Bystander hyperactivation of preimmune CD8+ T cells in chronic HCV patients. *Elife* **4,** (2015).
140. Martin, B. *et al.* Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *J. Hepatol.* **61,** 538–543 (2014).
141. Lawitz, E. *et al.* Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. *The Lancet* **384,** 1756–1765 (2014).
142. Mathew, R. *et al.* BTB-ZF factors recruit the E3 ligase cullin 3 to regulate lymphoid effector programs. *Nature* **491,** 618–621 (2012).
143. Spaan, M. *et al.* Frequencies of Circulating MAIT Cells Are Diminished in Chronic HCV, HIV and HCV/HIV Co-Infection and Do Not Recover during Therapy. *PLoS ONE* **11,** e0159243 (2016).
144. Bolte, F. J. *et al.* Intra-Hepatic Depletion of Mucosal-Associated Invariant T Cells in Hepatitis C Virus-Induced Liver Inflammation. *Gastroenterology* **153,** 1392–1403.e2 (2017).
145. Dias, J. *et al.* Chronic hepatitis delta virus infection leads to functional impairment and severe loss of MAIT cells. *J. Hepatol.* **71,** 301–312 (2019).

146. Horowitz, A. *et al.* Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. *Sci Transl Med* **5,** 208ra145 (2013).
147. Strauss-Albee, D. M. *et al.* Human NK cell repertoire diversity reflects immune experience and correlates with viral susceptibility. *Sci Transl Med* **7,** 297ra115 (2015).
148. Lu, Y. *et al.* Systematic Analysis of Cell-to-Cell Expression Variation of T Lymphocytes in a Human Cohort Identifies Aging and Genetic Associations. *Immunity* **45,** 1162–1175 (2016).
149. Oliviero, B. *et al.* Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. *Gastroenterology* **137,** 1151–60–1160.e1–7 (2009).
150. Serti, E. *et al.* Successful Interferon-Free Therapy of Chronic Hepatitis C Virus Infection Normalizes Natural Killer Cell Function. *Gastroenterology* **149,** 190–200.e2 (2015).
151. Nattermann, J. *et al.* Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C. *Gut* **55,** 869–877 (2006).
152. Deterding, K. *et al.* Improvement of liver function parameters in advanced HCV-associated liver cirrhosis by IFN-free antiviral therapies. *Aliment. Pharmacol. Ther.* **42,** 889–901 (2015).
153. Spaan, M. *et al.* Immunological Analysis During Interferon-Free Therapy for Chronic Hepatitis C Virus Infection Reveals Modulation of the Natural Killer Cell Compartment. *J. Infect. Dis.* **213,** 216–223 (2015).
154. Hengst, J. *et al.* Direct-Acting Antiviral-Induced Hepatitis C Virus Clearance Does Not Completely Restore the Altered Cytokine and Chemokine Milieu in Patients With Chronic Hepatitis C. *J. Infect. Dis.* **214,** 1965–1974 (2016).
155. Carlin, A. F. *et al.* Temporal dynamics of inflammatory cytokines/chemokines during sofosbuvir and ribavirin therapy for genotype 2 and 3 hepatitis C infection. *Hepatology* **62,** 1047–1058 (2015).
156. Gorin, J.-B. *et al.* Plasma FABP4 is associated with liver disease recovery during treatment-induced clearance of chronic HCV infection. *Nature Publishing Group* **10,** 2081–10 (2020).
157. Aregay, A. *et al.* Elimination of hepatitis C virus has limited impact on the functional and mitochondrial impairment of HCV-specific CD8+ T cell responses. *J. Hepatol.* **71,** 889–899 (2019).
158. Wieland, D. *et al.* TCF1+ hepatitis C virus-specific CD8+ T cells are maintained after cessation of chronic antigen stimulation. *Nat Commun* **8,** 15050–13 (2017).
159. Ghosh, A. *et al.* Persistent gamma delta T-cell dysfunction in chronic HCV infection despite direct-acting antiviral therapy induced cure. *J. Viral Hepat.* (2019). doi:10.1111/jvh.13121
160. Ravens, S. *et al.* Human γδ T Cell Receptor Repertoires in Peripheral Blood Remain Stable Despite Clearance of Persistent Hepatitis C Virus Infection by Direct-Acting Antiviral Drug Therapy. *Front Immunol* **9,** 510 (2018).
161. Hengst, J. *et al.* Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. *Eur. J. Immunol.* **46,** 2204–2210 (2016).
162. Langhans, B. *et al.* Increased peripheral CD4+ regulatory T cells persist after successful direct-acting antiviral treatment of chronic hepatitis C. *J. Hepatol.* **66,** 888–896 (2017).
163. Strunz, B. *et al.* Chronic hepatitis C virus infection irreversibly impacts human natural killer cell repertoire diversity. *Nat Commun* **9,** 2275–12 (2018).

164. Hegde, P. *et al.* Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. *Nat Commun* **9,** 2146–12 (2018).
165. Brenchley, J. M. *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12,** 1365–1371 (2006).
166. Rainer, F. *et al.* Soluble CD163 and soluble mannose receptor predict survival and decompensation in patients with liver cirrhosis, and correlate with gut permeability and bacterial translocation. *Aliment. Pharmacol. Ther.* **47,** 657–664 (2018).
167. Moghadamrad, S. *et al.* Attenuated portal hypertension in germ-free mice: Function of bacterial flora on the development of mesenteric lymphatic and blood vessels. *Hepatology* **61,** 1685–1695 (2015).
168. Riva, A. *et al.* Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. *Gut* **67,** 918–930 (2018).
169. Ruiz-Alcaraz, A. J. *et al.* Characterization of human peritoneal monocyte/macrophage subsets in homeostasis: Phenotype, GATA6, phagocytic/oxidative activities and cytokines expression. *Nature Publishing Group* **8,** 12794–14 (2018).
170. Tang, X.-Z. *et al.* IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *J. Immunol.* **190,** 3142–3152 (2013).
171. Mueller, S. N. & Mackay, L. K. Tissue-resident memory T cells: local specialists in immune defence. *Nat. Rev. Immunol.* **16,** 79–89 (2016).
172. Yasumoto, K. *et al.* Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. *Cancer Res.* **66,** 2181–2187 (2006).
173. Wang, H. *et al.* IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection. *Sci Immunol* **4,** eaaw0402 (2019).
174. Hickey, D. K., Patel, M. V., Fahey, J. V. & Wira, C. R. Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: stratification and integration of immune protection against the transmission of sexually transmitted infections. *J. Reprod. Immunol.* **88,** 185–194 (2011).
175. Nancy, P. & Erlebacher, A. T cell behavior at the maternal-fetal interface. *Int. J. Dev. Biol.* **58,** 189–198 (2014).
176. Vento-Tormo, R. *et al.* Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* **563,** 347–353 (2018).
177. Borsellino, G. *et al.* Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* **110,** 1225–1232 (2007).
178. Orrù, V. *et al.* Genetic variants regulating immune cell levels in health and disease. *Cell* **155,** 242–256 (2013).
179. Vacca, P. *et al.* CD34+ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. *Proc Natl Acad Sci U S A* **108,** 2402–2407 (2011).
180. Sojka, D. K. *et al.* Cutting Edge: Local Proliferation of Uterine Tissue-Resident NK Cells during Decidualization in Mice. *J. Immunol.* **201,** 2551–2556 (2018).
181. Keskin, D. B. *et al.* TGFbeta promotes conversion of CD16+ peripheral blood NK cells into CD16- NK cells with similarities to decidual NK cells. *Proc Natl Acad Sci U S A* **104,** 3378–3383 (2007).
182. Carlino, C. *et al.* Recruitment of circulating NK cells through decidual tissues: a possible mechanism controlling NK cell accumulation in the uterus during early pregnancy. *Blood* **111,** 3108–3115 (2008).

183. Dunn, C. L., Critchley, H. O. D. & Kelly, R. W. IL-15 regulation in human endometrial stromal cells. *J. Clin. Endocrinol. Metab.* **87,** 1898–1901 (2002).
184. Wilkens, J. *et al.* Uterine NK cells regulate endometrial bleeding in women and are suppressed by the progesterone receptor modulator asoprisnil. *J. Immunol.* **191,** 2226–2235 (2013).
185. Deng, K. *et al.* Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature* **517,** 381–385 (2015).
186. Rongvaux, A. *et al.* Development and function of human innate immune cells in a humanized mouse model. *Nature Biotechnology* **32,** 364–372 (2014).
187. Blois, S. M. *et al.* A pivotal role for galectin-1 in fetomaternal tolerance. *Nat. Med.* **13,** 1450–1457 (2007).
188. Zhu, C. *et al.* The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat. Immunol.* **6,** 1245–1252 (2005).
189. Huhn, O. *et al.* Distinctive phenotypes and functions of innate lymphoid cells in human decidua during early pregnancy. *Nat Commun* **11,** 381–14 (2020).
190. Okada, H. *et al.* Progesterone enhances interleukin-15 production in human endometrial stromal cells in vitro. *J. Clin. Endocrinol. Metab.* **85,** 4765–4770 (2000).
191. Thijssen, V. L. & Griffioen, A. W. Galectin-1 and -9 in angiogenesis: a sweet couple. *Glycobiology* **24,** 915–920 (2014).
192. Antonioli, L., Pacher, P., Vizi, E. S. & Haskó, G. CD39 and CD73 in immunity and inflammation. *Trends Mol Med* **19,** 355–367 (2013).